

PART I - ADMINISTRATIVE

Section 1. General administrative information

Title of project

Assessment of Captive Broodstock Technology

BPA project number	9305600
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Contract renewal date (mm/yyyy)	08/2000
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Multiple actions? (indicate Yes or No)	no
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Business name of agency, institution or organization requesting funding

National Marine Fisheries Service

Business acronym (if appropriate)	NMFS
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Proposal contact person or principal investigator:

Name	Dr. Penny Swanson
Mailing address	NFSC - 2725 Montlake Blvd. East
City, ST Zip	Seattle, WA 98112
Phone	206-860-3282
Fax	206-860-3267
Email address	penny.swanson@noaa.gov

NPPC Program Measure Number(s) which this project addresses

Measure 7.4D.1 in the NPPC F & W Program,

FWS/NMFS Biological Opinion Number(s) which this project addresses

Steelhead Biop

Other planning document references

Task 4.1.c in the NMFS Proposed Snake River Recovery Plan

Short description

Improve effectiveness and assess risks of captive broodstock programs as a tool for recovery of depleted salmon stocks

Target species

Pacific salmon *Oncorhynchus* sp.

Section 2. Sorting and evaluation

Subbasin

Evaluation Process Sort

CBFWA caucus		CBFWA eval. process		ISRP project type	
X one or more caucus		If your project fits either of these processes, X one or both		X one or more categories	
x	Anadromous fish	x	Multi-year (milestone-based evaluation)		Watershed councils/model watersheds
	Resident Fish		Watershed project eval.	x	Information dissemination
	Wildlife				Operation & maintenance
					New construction
				x	Research & monitoring
					Implementation & mgmt
					Wildlife habitat acquisitions

Section 3. Relationships to other Bonneville projects

Umbrella / sub-proposal relationships. List umbrella project first.

Project #	Project title/description

Other dependent or critically-related projects

Project #	Project title/description	Nature of relationship
9202200	Wild smolt physiology/behavior	Collaborate by evaluating smoltification of experimental groups of spring chinook salmon.

Section 4. Objectives, tasks and schedules

Past accomplishments

Year	Accomplishment	Met biological objectives?
1994-1996	Compared reproductive performance of sockeye salmon reared in either fresh or salt water.	Yes.
1994-1996	Compared effectiveness of biodegradable and nonbiodegradable GnRH implants for induction of ovulation and spermiation in sockeye salmon	Yes. Hormone implants effectively advanced and synchronized spawning without impairing gamete viability.
1993-1996	Examined the relationship between body fat levels and early male maturity in spring chinook salmon	Yes. Increased body fat levels were correlated with the onset of maturity at age 2.
1995-1997	Examined independent and interactive effects of growth rate and body fat levels on onset of maturity in male spring chinook salmon	Yes. Growth or body size is predominant factor affecting maturation at age 2 in males, and body fat levels are secondary

1997-present	Examine relationship between growth rate or ration level on onset of age of maturity in male spring chinook salmon	Ongoing.
1994-1998	Determine critical period of olfactory imprinting in sockeye and spring chinook salmon	Yes. Experiments were conducted but results were inconclusive.
1994-1997	Tested improved broodstock diets for sockeye salmon	Yes. Diets supplemented with krill and vitamins reduced embryonic deformities but had no effect on egg quality or age of maturity
1995-1997	Tested various dietary lipid levels for effects on reproductive performance in sockeye salmon	Yes. Highest levels of dietary fat reduced appetite, but no effect was observed on gamete quality or age of maturity
1996-1998	Developed/validated bioencapsulation procedures to deliver antibiotics to first feeding salmon fry	Yes. Therapeutic levels of antibiotic in fry were obtained with bioencapsulated antibiotic
1995-1998	Tested live food diets for sockeye salmon fry	Yes. Fry fed live food did not differ from those fed commercial fry diets in the type of live food selected in later stages of development.
1996-1997	Evaluated reproductive behavior of chinook salmon in artificial spawning stream	Yes.
1995-1996	Compared reproductive success of captively reared and sea ranched coho salmon	Yes.
1994-1998	Determined effects of rearing sockeye salmon at either 8 or 12 C on growth, age of maturity, smoltification and gamete quality	Yes. No negative effect of rearing temperature on smoltification or gamete quality was found. A significant effect of temperature was found on growth and age of maturity with 12 C increasing growth and decreasing age of maturity.
1994-1998	Development of methods to measure the nonspecific immune functions of salmonids	Yes. Developed panel of assays to assess immune functions of salmonid broodstock reared in captivity.
1994-1998	Measurement of cellular immune functions of sockeye salmon throughout their entire life cycle.	Yes. Completed first quantitative profile of immune functions of sockeye salmon reared at 8 °C and 12 °C.
1994-1998	Quantification of the effect of rearing temperature on the ability of sockeye salmon to produce antibody response.	Yes. An ELISA for fish antibody was developed to measure the specific antibody response of salmon to a selected protein, pathogen or vaccine. Fish reared on differed tempertures

		showed different antibody response.
1994-1998	Quantification of the effect of smoltification of sockeye salmon on immune functions which are important for disease resistance.	Yes. A quantitative profile of immune functions of sockeye salmon reared on either 8 or 12 C during smoltification was completed in 1997.
1997-present	Quantification of effects of growth rate on immune functions of chinook salmon.	Ongoing. Results suggest that cellular mediated immunity is impaired when fish are reared on high growth rates.
1997-present	Test azithromycin for reducing mortality due to BKD in sockeye salmon	Yes. Sample analysis is ongoing, but results suggest mortality was reduced with antibiotic and gamete viability was not impaired.
1998-present	Test azithromycin for reducing mortality due to BKD in sockeye salmon	Ongoing.
1998-present	Examine mate preference in chinook salmon	Ongoing. Data suggests females prefer larger males.
1994	Established quantitative genetic experimental design	Yes
1995	Released to sea 257,000 fish marked with family specific coded wire tags	Yes
1998	Completed genetic analysis of juvenile body morphometry in base population	Yes
1998	Cultured 2-, 3-, and 4-year old PIT tagged fish from the same cohort to maturity	Yes
1998	Established experimentally inbred (1 generation, 2 levels of inbreeding) and control lines of progeny	Yes

Objectives and tasks

Obj 1,2,3	Objective	Task a,b,c	Task
1.1	Determine the effect of growth rate on early male maturation, smoltification and immunocompetence in spring chinook salmon containing low levels of body fat		
1.2	Determine the effects of reduced temperature and ration during winter		

	months on early male maturation in chinook salmon		
1.3	Determine the effects of constant vs. Variable dietary protein intake on growth, body conformation, natural spawning success, spawning behavior, and reproductive performance of chinook salmon		
2.1	Monitor nonspecific and specific immune functions of groups of spring chinook salmon reared on high protein low fat diets reduced temperature and ration during winter months (obj. 1.2)		
2.2	Test effectiveness of azithromycin as an emergency treatment against acute BKD in chinook salmon		
2.3	Test effects of long term anti-BKD prophylactic treatment regimens with erythromycin on disease incidence, gonad development, and gamete quality in fall chinook salmon		
3.1	Determine effects of exercise on body composition, morphology and breeding success of captive reared chinook salmon		
3.2	Determine relative effects of natural emergence, remote site incubation and fry releases on the early growth of chinook salmon juveniles		
3.3	Compare natural breeding success of freshwater- and sea water-reared chinook salmon		
4.1	Determine quantitative genetic consequences of inbreeding depression on fitness of chinook salmon population		

Objective schedules and costs

Obj #	Start date mm/yyyy	End date mm/yyyy	Measureable biological objective(s)	Milestone	FY2000 Cost %
1.1	10/1997	10/1999	Rear chinook salmon from first feeding to 2 years of age on 7 dietary treatments, collect samples for monitoring growth, smoltification and maturation	Complete sample collection	3.8
	10/1999	12/2000	Evaluate effects of dietary treatments on growth physiology, smoltification, age of maturity, size and morphology	Analyze samples, data and prepare manuscript	11.5
1.2	10/1998	10/2000	Rear chinook salmon from first feeding to 2 years of age on 4 dietary/temperature regimes, collect samples for monitoring growth, smoltification and maturation	Complete sample collection	9.2
	10/2000	12/2001	Evaluate effects of treatments on growth physiology, smoltification, age of maturity, size and morphology	Analyze samples, data and prepare manuscript	4.0
1.3	08/1998	04/2003	Rear chinook salmon to maturation on constant versus variable dietary protein:energy intake	Rear fish to maturity and collect samples for body morphology and composition	5.6
			Evaluate effects of diets on reproductive performance, growth, morphology and behavior of chinook salmon	Analyze samples, behavior of adult fish and data, and write report	
2.1	06/1999	11/2000	Examine effects of growth and nutrition on immune function in chinook	Quantify effects of diet and growth regime on specific	7.6

			salmon	and nonspecific immunity, collect samples, analyze data and write report	
2.2	07/1998	12/1999	Test effectiveness of azithromycin as an emergency treatment for BKD in chinook salmon	Measure mortality, onset of BKD and presence of pathogen in treatment groups, analyze data, write reports	9.8
2.3	05/1999	08/2002	Test effects of long term treatment with erythromycin on disease incidence, gonad development, and gamete quality in fall chinook salmon	Rear fish, measure mortality, presence of pathogen, onset of BKD, gamete quality and survival of offspring to first feeding. Analyze data and write report	9.8
3.1	06/2000	09/2000	Culture of chinook salmon in high and low velocity vessels	Produce mature adults	2
	09/2000	12/2000	Body composition and morphology of exercised and non-exercised salmon	Analyze morphological characters, and body composition, and complete report	2
	08/2000	10/2000	Breeding behavior and reproductive success of exercised and non-exercised salmon	Collect spawning behavior and egg deposition data, analyze spawning behavior data and complete report	6.6
3.2	09/2000	11/2000	Artificially spawn captively reared chinook salmon and thermally mark otoliths	Produce 5000 viable salmon embryos with thermal marks	1.1
	01/2001	03/2001	Survival of embryos in remote site incubators,	Produce suitable incubation	2

			heath trays, and gravel	environments to achieve high survival	
	03/2001	05/2001	Growth of juvenile salmon from three reintroduction strategies	Collect growth data, decode otoliths, and complete report	3.1
3.3	08/2000	10/2000	Breeding behavior and success of fresh and salt water reared salmon	Collect spawning behavior and egg deposition data	1.6
	10/2000	05/2001	Breeding behavior and success of fresh and salt water reared salmon	Collect spawning behavior data and assist in report	1.2
4.1	11/1994	12/2005	Determine quantitative genetic consequences of inbreeding depression on fitness of chinook salmon population	Two generations of inbreeding completed	19
				Total	100

Schedule constraints:

Completion date:12/2005

Section 5. Budget

FY99 project budget (BPA obligated): | \$1,300K

FY2000 budget by line item

Item	Note	% of total	FY2000 (\$)
Personnel		14.55	190600
Fringe benefits		3.7	48600

Supplies, materials, non-expendable property		11.62	152200
Operations & maintenance		2.9	38000
Capital acquisitions or improvements (e.g. land, buildings, major equip.)		1.03	13500
NEPA costs			
Construction-related support			
PIT tags	# of tags: 2000	.46	6000
Travel		1.26	16500
Indirect costs		12.72	166700
Subcontractors: (contracts & grants)			
Univ. Washington		23.7	310500
Wash. Dept. Fish and Wildl.		.84	11000
Pac. States Mar. Fish. Com.		9.9	129700
Nickelson Drilling		.31	4000
Alaska Marine Refrig.		.23	3000
NW Indian Fish. Comm.		3.21	42000
Frank Orth & Assoc.		6.9	90000
Equipment Maintenance		1.15	15000
Univ. Idaho		5.6	73000
Other Cost			

TOTAL BPA REQUESTED BUDGET 1,310,300

Cost sharing

Organization	Item or service provided	% total project cost (incl. BPA)	Amount (\$)

Total project cost (including BPA portion)

Outyear costs

	FY2001	FY02	FY03	FY04
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Total budget	1,400,000	1,300,000	1,200,000	1,000,000
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Section 6. References

Watershed ?	Reference

PART II - NARRATIVE

Section 7. Abstract

In response to Task 4.1.c in the NMFS Proposed Recovery Plan and to Measure 7.4D.1 in the NPPC F & W Program, this research project develops information needed to overcome some of the problems that limit the yield of viable offspring from Pacific salmon stocks reared in captivity, and assesses some of the genetic consequences of captive broodstock programs. While basic fish husbandry techniques are well established and widely used for rearing juvenile salmonids from gametes collected from returning adults, and domesticated stocks of salmonids in the commercial aquaculture industry, numerous problems have persisted when rearing wild stocks of Pacific salmon in captivity throughout the life-cycle. These problems include poor survival of adults to spawning, poor quality gametes, and abnormal seasonal timing of spawning. The success of captive broodstock programs for stock restoration purposes is largely dependent on producing a high yield of offspring that do not differ substantially from the founder stock in genetics, behavior, appearance, or physiology. Solutions to the problems encountered by broodstock programs are needed to maximize the effectiveness of these programs as rehabilitative tools. In addition, the reproductive success of captively-reared fish must be evaluated to determine if release of captively-reared adults is a viable strategy. The overall goals of this project are 1) to develop diets, rearing regimes, hatchery practices, and drug therapies that improve survival of adults to spawning, gamete quality, and viability of offspring and that can be applied to captive broodstock programs for depressed stocks of Pacific salmon; 2) to assess quantitative genetic risks of captive broodstock programs to natural populations; and 3) to develop reintroduction strategies for captively-reared fish. Results from this research will be published in peer-reviewed journals, annual reports and scientific meetings.

Section 8. Project description

a. Technical and/or scientific background

INTRODUCTION

One of the current barriers to restoration of many depleted stocks of Pacific salmon (*Oncorhynchus* spp.) in the Columbia River Basin is the availability of suitable numbers of juveniles for supplementation. The Northwest Power Planning Council's Columbia River Basin Fish and Wildlife Program was recently amended to include development and implementation of captive broodstock technology to aid recovery of salmon stocks (Phase II; Measure VI.B.6.A.2). Captive broodstock programs are a form of artificial propagation. However, they differ from traditional hatchery programs in one important respect: fish are cultured in captivity for their entire life cycle.

Like salmon hatchery programs, however, captive broodstock programs are not without problems and risks to natural salmon populations. Captive broodstock programs can sustain high mortality, which may increase a population's risk of extinction if the captive component is a substantial fraction of this population. Rearing systems must be designed and operated to minimize the risk of loss due to disease, poor reproductive performance of adult fish, and poor survival of offspring once released into the native habitat. Additional risks include genetic change imposed on a population by a captive broodstock program, genetic interaction between captive and natural fish in the wild, and ecological impacts of releases of captive fish on natural populations.

Although captive broodstock technology has been widely applied to other vertebrates, its application to restoration of depleted stocks of Pacific salmon is in its infancy. Recent experience with captive broodstock programs has indicated that substantial problems exist with: 1) poor survival of fish to spawning; 2) inappropriate timing of sexual maturation; 3) poor quality gametes; and 4) reintroduction of captively-reared fish. In addition, there are not adequate therapies available for treating fish once disease, particularly bacterial kidney disease, occurs, and the reproductive success of captively reared adult fish and the survival of offspring from captively reared fish when released into the wild is unknown. We are conducting and propose to continue a multi-faceted research program designed to overcome these problems by establishing rearing regimes, diets, and environmental conditions that lead to appropriate body coloration, size, and timing of development through the life cycle, and high survival rates of broodstock and their offspring. Our literature review also indicated that little research has been done that relates directly to the genetic consequences of captive broodstock programs for Pacific salmon. Therefore, we are conducting quantitative genetic experiments to characterize some important potential effects of captive culture on the genetic constitution of the cultured population. The experiments require access to one or more large source populations and much of the rearing capacity of a single facility for up to four fish generations.

The project has three primary goals: 1) to develop standard, efficient hatchery practices for rearing captive Pacific salmon broodstock that will yield the greatest number of high-quality offspring (those that are as similar to the founder stock as possible), 2) to determine the genetic consequences of captive broodstock programs for natural salmon populations, and

3) to evaluate the reproductive success of captively-reared adult fish compared to wild fish and the viability of captively-reared offspring upon release . The work proposal has been divided into four major research elements: 1) effects of diet and growth on age of maturity, adult morphology, smoltification, body coloration, and gamete quality ; 2) fish health; 3) reproductive ecology of captively-reared adult salmon; and 4) research on quantitative genetic consequences of captive broodstock programs for Pacific salmon populations.

Element 1- Effects of Growth and Diet on Age of Maturity, Smoltification, Body Coloration, and Gamete Quality in Chinook and Sockeye Salmon

One critical problem for captive rearing of chinook salmon is loss of fish due to early sexual maturation of males. In many salmonid species, males may mature early relative to females, with the incidence varying among species, stocks, and rearing conditions for cultured fish. The chinook salmon has a high degree of plasticity in its life cycle compared to other Pacific salmon species. Early, or precocious, male maturation can occur at several stages of the life cycle. Jacking rates as high as 90% have been observed (Hard et al., 1985), although most chinook stocks exhibit rates around 5-15% (Heath, 1992). In a captive broodstock program, it is undesirable to produce mature males at a time when females of the same stock are not mature. Although this milt from early maturing males could be cryopreserved, this technique is not yet sufficiently reliable to obtain consistently high quality sperm. In addition, selective mortality of precocious males could reduce the effective breeding population size (N_e) of a captive broodstock. Thus, there is a critical need to develop methods to minimize precocious male maturation in captive broodstock programs for endangered fish species.

The time of sexual maturation is controlled by genetic, abiotic (e.g., photoperiod, temperature, salinity) and biotic (e.g., diet, growth rate, energy stores) factors. The relative importance of these factors and how they interact are poorly understood. Because genetic selection should be minimized in a captive broodstock program for depleted stocks, rearing strategies which minimize expression of the trait should be developed. Research to date, primarily from Atlantic salmon, indicates that growth rate, size and levels of stored energy at specific times of year, or critical periods of the life cycle are important factors affecting the incidence of precocious maturation. It may be possible to minimize the incidence of precocious male maturation through alteration in rearing conditions, growth rates, and diet. However, before methods that minimize the rate of precocious male maturation can be developed, research is necessary to determine how stored energy levels (body fat content), growth rates, or rates of energy deposition at critical developmental stages either permit or prevent the onset of maturation in chinook salmon.

Growth rate and body fat levels

We are attempting to develop diets and growth regimes that sustain somatic growth and provide sufficient stored energy for appropriate life-cycle transitions, development of gametes in adult fish, and achieve target size for adult fish. To develop a diet and growth regime that minimizes early maturation of male spring chinook salmon, we are engaged in two key areas of investigation. In initial studies we manipulated body fat levels through diet and found a significant positive correlation of percent of males maturing at 2-years of age with body fat levels. Second, we investigated whether reduced growth during the autumn/winter period can reduce the number of males maturing at one or two years of age and found that severe ration restriction reduces early male maturity, but ration reduction during a 2 or 4 month period

during the winter was insufficient to reduce the rate of maturation of males at 2 years of age. Results from this study are consistent with previous studies in Atlantic salmon which indicated that severe ration restriction can reduce the incidence of early male maturity. However, a protocol which utilizes severe ration restriction may have undesirable effects on smoltification, maturation of females in subsequent years, and ability of managers to achieve target sizes for adults. One of the problems with previous research in this area is that effects of size, growth rate, and rate of energy deposition could not be distinguished. Therefore we conducted a study varying ration and fat levels and found that size or growth rate was the primary factor affecting the rate of maturation of 2-year old fish, and fat level was a secondary factor (Shearer et al. 1997; Silverstein et al. 1997).

The aim of ongoing experiments is to more precisely determine how rapidly male chinook salmon can be grown from first feeding to two years of age without increasing the incidence of maturation. Since growth rate has been shown to affect both smoltification and immunocompetence we are examining the effects of growth on these factors. Fish are being reared on high protein, low fat diets to reduce the effects of body fat levels on early male maturity. Once a threshold growth rate for early male maturity is established, we propose to conduct experiments whereby we reduce growth during the winter months using both ration and water temperature in an attempt to reduce growth below threshold levels only during critical months when the physiological commitment to mature is made. In these studies of the effects of growth and body energy stores on early male maturity we are monitoring growth, reproductive development, smoltification, immune function (see Element 2), and body morphology.

Variable dietary protein:energy levels

In a second part of our studies on growth in salmonids, we are investigating the effects of variable dietary protein :energy ratios on age of maturity and adult morphology in chinook salmon. The natural diet of chinook salmon during the post-juvenile marine phase of its life cycle consists mainly of herring, anchovy, other small fish, and squid. When this diet is expressed in terms of proximate composition, protein, lipid, and ash (bones) fractions constitute more than 95% of the diet. If a feed were formulated to mimic the natural diet of chinook salmon in the ocean, it would be approximately 50% protein, 32% lipid, and 10% ash (Higgs et al., 1995). Feeds used to rear post-juvenile chinook salmon in captivity, e.g., net-pen farming, have a proximate composition of 45% protein, 18-22% lipid, and 12% ash, with the remaining constituents being crude fiber (~2%) and nitrogen-free extract (soluble carbohydrates) as the remainder. Farmed chinook salmon fed diets containing higher levels of lipid throughout the seasons accumulate excessive amounts of body fat, mainly during the period of declining day length. In contrast, the natural diet of chinook salmon during the early phases of marine life history, e.g., copepods, decapods, amphipods, and euphausiids, contains much higher dietary lipid levels than pelleted feeds. The protein and lipid levels of each average 52% and 37% for copepods, 73% and 12% for decapods, 47% and 24% for amphipods, and 54% and 22% for euphausiids, respectively (Higgs et al., 1995). Squid contain 78% protein and 13% lipid, while gastropods average 44% protein and 22% lipid. Despite this high lipid diet, wild post-juvenile chinook salmon do not accumulate excessive levels of body fat, most likely a consequence of activity level and caloric intake.

The natural diets of juvenile salmonids in freshwater consist mainly of aquatic and terrestrial insects. Many published studies have documented the natural diet of juvenile salmonids, both in terms of prey and in terms of nutrient intake, beginning with Embury and Gordon (1924) and most recently reviewed in depth by Higgs et al. (1995). These studies

conclusively show that the proximate composition of natural diets of juvenile salmonids is approximately 45% protein and 15-17% lipid. Although the natural diet of juvenile chinook salmon in freshwater consists of insects having lipid levels ranging from 2-39%, on a dry weight basis, the main four groups of insects consumed by juvenile chinook average 53% protein and 16.5% lipid, similar to the composition of freshwater copepods and small fishes. Like wild post-juveniles, juvenile chinook are distinctly different from hatchery-reared chinook in body fat deposits, particularly in the amount of fat in visceral stores. These differences are greater among stream-type juvenile chinook than among ocean-type.

The body conformation of chinook salmon reared in captive broodstock programs differs from that of wild fish. The main difference in body conformation is length to girth ratio, with captively-reared fish having a lower length to girth ratio, meaning that they tend to be shorter and fatter. This difference is presumably related to dietary energy intake and differences between wild and captive fish in activity level. Dietary energy intake is similar between wild and captively-reared fish, when expressed in terms of proximate composition, or percentage of the diet as protein and lipid. However, dietary energy intake is a function of the proximate composition of the diet as well as feed intake. Wild fish consume less food during certain periods of the year than do captively-reared fish. Rarely are chinook salmon with full stomachs captured in the fisheries, except in nearshore areas where fish are feeding heavily during the months before entering freshwater to spawn. Recent BPA-funded studies with chinook salmon (W. W. Dickhoff, 1997) have identified annual variations in levels of the metabolic hormones IGF-1 and growth hormone which provides a metabolic rationale for numerous observations made for over 40 years concerning the higher level of whole body lipid in hatchery-reared fish compared to wild salmon. During periods of declining day length, circulating levels of these hormones are low and protein synthesis rates in the body are reduced. Fish convert both dietary protein and lipid into stored body fat during these periods. In contrast, when day lengths increase, body metabolism patterns change, with increases in protein synthesis rates and lypolysis, resulting in lower percentage whole body fat levels. When feeding levels are adjusted to correspond with this pattern of anabolism and catabolism, survival to hatchery return increases. With respect to changing dietary requirements as fish grow and develop, it is well known that the scope for growth of Pacific salmon decreases with fish size (Brett, 1979). Thus, the protein needs of salmon, expressed as a percentage of dietary metabolic energy, decrease with fish size. McCallum (1985) reported that juvenile chinook salmon required 1.3-1.6 g protein per kg fish per day at the maintenance level, e.g. no growth. The amount of protein intake to support weight gain varies with fish size and dietary energy intake but growth increases linearly in juvenile salmonids consuming between 6 and 18 g protein per kg fish per day (Fairgrieve, 1992). Therefore, we are investigating the effects of a diet of variable protein and energy on growth, age of maturity, adult morphology and reproductive performance in spring chinook salmon.

Element 2- Fish Health

To reduce mortality due to disease in captive broodstocks there are two strategies: employ hatchery practices, environmental conditions, and feeding regimes which maintain good fish health and minimize disease transmission, and utilize effective drug therapies once disease outbreaks occur. In 1987, the Pacific Northwest Fish Health Protection Committee ranked bacterial kidney disease (BKD) as the major deterrent to the successful culture of

salmonids in the Pacific Northwest. In 1993 - 1994 this disease was responsible for catastrophic losses of endangered Redfish Lake sockeye salmon being held as captive broodstock and many other cases of severe mortality among Snake River Chinook salmon broodstocks have been reported. We are conducting research in two areas in an attempt to reduce mortality due to disease in captive broodstock. First, we are testing new drug therapies for treating BKD and evaluating possible toxicity of erythromycin. Second, we are investigating the effects of nutrition and rearing temperature on immune function. In previous funded periods, we developed a range of standard assays to assess both humoral and cellular mediated immunity and have used these assays to assess immunocompetence in experimental fish reared on different water temperatures or under a range of growth conditions. Our results suggested that high ration (growth rate) reduces immunocompetence. We propose to extend those studies to more thoroughly evaluate the effects of high growth on immune function and disease resistance.

Drug Therapies

Presently there is no vaccine available to protect salmon from infections with *Renibacterium salmoninarum*, the causative bacterium of BKD. Erythromycin has been the primary antibiotic used by fish culturists in an attempt to prevent and control *R. salmoninarum* (Elliott et al. 1989), administered orally through feed and by injection of maturing adults. However, while use of this drug results in short term health improvement of infected fish, it fails to completely eliminate the disease and symptoms often return upon cessation of treatment. Compounding the problem is that transmission of the disease occurs both horizontally and vertically. Prophylactic erythromycin treatment of spawning females or its use during water hardening of eggs does seem to reduce some of the vertical transmission of *R. salmoninarum* to eggs (Evelyn et al. 1986, Bullock and Stuckey 1986, Groman 1983), but in general this practice has not led to satisfactory results. In spite of these shortcomings, prophylactic administration of erythromycin has become increasingly common in recent years, particularly in captive broodstock programs involving ESA listed stocks of sockeye and chinook salmon. This use is not without cost. Results of preliminary studies with Lake Wenatchee sockeye salmon suggest that erythromycin may have a negative effect on gamete viability. Recent experiences at Manchester Field Station with Catherine Creek, Lostine River, and Lemhi River spring chinook salmon have shown currently mandated treatment regimens may elicit fatal toxicity reactions. In light of these observations of low efficacy of erythromycin combined with potential toxic and reproductive success effects during prolonged prophylactic use, there is a clear need for additional study in these areas.

Alternative antibiotics: azithromycin

Azithromycin is a promising candidate for use as a therapeutic and prophylactic treatment for BKD. This new macrolide antibiotic concentrates in polymorphonuclear leukocytes, macrophages and fibrocytes (Peters et al. 1992). *R. salmoninarum* has been shown to invade these cell types which in turn protect the organism from the host humoral immune system. (Bandin et al. 1993; Gutenberger et al. 1997).

Most antibiotics, including erythromycin, do not penetrate tissues well. After oral or parenteral administration, they are bound to serum protein and remain in extracellular spaces. To the contrary, azithromycin is rapidly absorbed in tissues and is widely distributed at higher concentrations in cells than in plasma or in serum, with a longer active half life (Peters et al. 1992). Azithromycin has been shown to be effective in reducing the intracellular viabilities of nearly all invasive bacterial species tested. In general, azithromycin is more effective than erythromycin against Gram-negative bacteria, but marginally less active against Gram-positive organisms (Peters et al. 1992). However, the latter is of doubtful clinical significance

because of the higher and longer-acting tissue concentrations that can be achieved with azithromycin. This view is supported by reports that show that azithromycin is somewhat more effective than erythromycin in reducing intracellular (invasive) enteric pathogens that were phagocytized by neutrophils (Rakita et al. 1994). The authors suggested that the concentration of azithromycin in neutrophils may be particularly useful in treating infections caused by invasive pathogens that multiply intracellularly in host cells. Numerous studies report greatly increased intracellular uptake and superior antibacterial activity of azithromycin over erythromycin in in vitro and in in vivo studies with animals other than fish (Peters et al. 1992). Other work compared the intracellular activity of azithromycin and erythromycin against an intracellular protozoan parasite, *Toxoplasma gondii*, and reported superior performance of azithromycin (Lode et al. 1996). Azithromycin accumulated readily and remained inside macrophages infected with the protozoan, interfering with growth of the parasite (Schwab et al. 1994). While there are no published studies demonstrating the efficacy of azithromycin in fish, its broad spectrum activity and ability to cross into tissues make it an important antimicrobial to test for its ability to control *R. Salmoninarum*.

Experiments conducted during previous funding periods have produced evidence that azithromycin may be a more effective antibiotic for treatment of BKD infections in captive broodstock. In 1996, we conducted trials to determine the efficacy of azithromycin in sockeye salmon by simulating an epizootic of BKD. Survival rates for the 3 treatment groups were as follows: azithromycin 57%, erythromycin 8%, and 0% for the control group. The non-infected sockeye salmon from the stocking population remained relatively healthy (<0.1% mortality). Necropsies in most fish revealed severe kidney lesions and other signs typically associated with clinical BKD. Currently we are examining approximately 10% of mortalities in all treatments using fluorescence antibody test (FAT) to confirm the presence of *Renibacterium salmoninarum* the causative agent of BKD.

To determine if multiple dosing with azithromycin would have toxic or deleterious effects on sockeye salmon or their offspring, 200 of the surviving azithromycin treated fish were randomly distributed into four 5 ft. diameter tanks (50/tank). They had all received the initial dose (30mg/kg fish/day for 14 days) of azithromycin at the beginning of the experiment. For this test, 2 tanks (100 fish) were fed 30 mg/kg fish/day for 14 days at 4 month intervals for a total of 4 treatments of azithromycin. The other 2 tanks were fed the non-medicated control feed during the treatment period. The four tanks received a Biodiet feed when they weren't being medicated. At maturity (3 years), 14 pairs (28 fish) were randomly selected from each treatment and were spawned using a 1/1 cross. We are currently testing all of the parents using FAT and ELISA. Overall, offspring viability has exceeded that observed by other captive-reared broodstock and there were very few anomalies. Preliminary results show there were no significant differences in survival of gametes to swim-up between fish treated once with azithromycin and those treated four times.

Attempts to show the effectiveness of azithromycin as a prophylactic therapeutic against BKD in chinook salmon have to date resulted in mixed results. In June 1998 a study was initiated to determine the effectiveness of azithromycin compared to erythromycin as a therapeutic agents against existing BKD. This experiment is presently ongoing. However, preliminary results show that therapeutic treatment with either erythromycin or azithromycin did offer protection in the form of longer survival. Bacterial loads of survivors has yet to be determined. Representative survivors as well as the uninfected control fish will be maintained in captivity to determine the effect of azithromycin treatment on sexual maturation, egg quality, and reproductive success, as outlined below (Objective 2.3). These experiments will

capitalize on the effort expended to initiate this subtask and will confirm the results observed previously in our studies of azithromycin in sockeye salmon.

Toxicity of prolonged or repeated use of erythromycin

As discussed in the introduction, results of preliminary studies with Lake Wenatchee sockeye salmon suggest that erythromycin may have a negative effect on gamete viability. Eyed egg survival for sockeye that were prophylactically treated with erythromycin up to four times per year and spawned during 1994 and 1995 ranged from 40-60% (Flagg et al. 1997). In contrast, for 1994-brood sockeye which had been treated with azithromycin once before smoltification and up to three additional times after transfer to seawater, egg viability ranged from 85-89% (Lee Harrell, personal communication). Ovarian inflammation in rainbow trout fed erythromycin thiocyanate has been reported (Piper 1961). However, no experimental data are currently available regarding the effects of long-term erythromycin prophylaxis on gonadal development and egg viability in any salmonid species. Controlled studies to determine whether erythromycin treatment has a negative effect on gamete quality are especially relevant to the success of captive rearing of ESA listed stocks.

In addition to these effects on gamete viability, recent experiences at Manchester Field Station with Catherine Creek, Lostine River, and Lemhi River spring chinook salmon have shown currently mandated treatment regimens may elicit fatal toxicity reactions. Typically, affected fish exhibit reddened skin in the cranial region, and swim erratically or quiver shortly before death, suggesting neurological involvement. Extensive liver damage has also been observed in moribund or dead fish. Experience has shown that terminating treatment alleviates the red-head condition. Similar toxic reactions to erythromycin have been reported in rainbow trout (Piper 1961, Warren 1963), but no data currently exist regarding the sensitivity of other salmonid species to this antibiotic, and it is not known if sensitivity increases with repeated treatments. Additional data are needed to fully document the conditions leading to the development of this syndrome.

Improving disease resistance

Flagg and Mahnaken (1995), in a review of the status of broodstock technology for Pacific salmon, highlighted the importance of understanding the complex relationships between fish nutrition, reproductive physiology, and fish health when developing fish husbandry practices. An ideal fish health management program for the captive propagation of sockeye or chinook salmon will have its foundation in a rearing environment that provides the most favorable conditions for growth and survival. Such conditions are very important because they help to maintain fish at their best physiological status to resist infection by pathogenic microorganisms, many of which are currently considered untreatable because of the lack of effective chemotherapeutics or vaccines.

We neither fully understand the host defenses of salmonids, nor whether they modulate with seasonal and life stage changes, or differing rearing conditions. To help answer those questions, researchers at the Western Fisheries Research Center in Seattle, Washington (U.S. Geological Survey) have developed a panel of hematological, immunological, and serological assays that can be used to measure the effects of different rearing conditions on the immune system of captive-reared salmonids. This panel of assays was used to measure nonspecific and specific immune functions of sockeye salmon from each of two temperature groups at various points during rearing. It was the first comprehensive monitoring of disease resistance systems in sockeye salmon for an entire lifecycle, and provided the methods and baseline information for future studies with captive-reared salmonids.

Bacterial kidney disease (BKD), caused by *Renibacterium salmoninarum*, is considered to be present worldwide among cultured and wild salmonid fishes (Fryer and Sanders 1981). Current control measures have proven ineffective in eliminating the disease (Elliott et al. 1989), and BKD can often be the limiting factor in the artificial propagation of salmonids. Chinook salmon are considered to be one of the most susceptible salmonid species to infection by the kidney disease bacterium (Bell 1987).

Captive propagation programs have been started to aid in the restoration of threatened or endangered spring chinook salmon stocks in Idaho and Oregon. These programs now rely on the capture of juvenile fish from wild or natural production areas of the Columbia River and Snake River Basins as sources of broodstock. There is frequent speculation that spring chinook salmon originating from wild or natural production areas have a lower prevalence of BKD than their hatchery counterparts. However, testing of wild and hatchery spring/summer chinook salmon smolts in the Snake River Basin has not shown lower prevalences and levels of *R. salmoninarum* infections in wild smolts (Elliott and Pascho 1994). These findings help to explain why some of the chinook salmon introduced into captive propagation programs have BKD.

Modifications of cultural practices that may exacerbate disease have been suggested as a way to reduce mortality from BKD (Klontz 1983), and among them, there has been considerable interest in the effect of diet composition. There is evidence that many of the specific and nonspecific host defenses of salmonids and other fishes are effected by specific dietary components (Reviewed by Landolt 1989; Blazer 1991, 1992; Waagbo 1994). Their effects on disease resistance to BKD, however, are not completely understood. One objective of the research being conducted under Project 93-56 is to measure the effect of growth rate on the ability of captive-reared chinook salmon to resist disease. The first measurements of specific and nonspecific immune parameters were completed during 1998. Preliminary results suggest the certain functions of a chinook salmon's phagocytic cells become impaired as the ration (and growth) is increased. These cells are the fish's first line of defense against bacterial pathogens such as *R. salmoninarum*, and any suggestion of their impairment by the diet only emphasizes the need for more information on the role of nutrition in fish health and disease resistance.

The failure to recognize deleterious effects by certain fish cultural practices may be part of the reason why vaccination of salmonids for BKD has been only marginally effective. Salmonids can respond to bacterins made of killed *R. salmoninarum* (Evelyn 1987; Paterson et al. 1981; Paterson et al. 1985; and McCarthy et al. 1984), but there is no clear correlation between a fish's ability to produce an antibody response to a bacterin and protection from BKD. Some researchers believe that the success of a vaccine may ultimately be dependent upon the salmonid fish species being immunized and the antigens of *R. salmoninarum* selected for a bacterin (Evelyn et al. 1988). However, genetic immunization with the new generation of DNA vaccines (Wolff et al. 1990) may hold promise for immunizing fish against BKD. Humoral and cellular immunity can be initiated by directly injecting the DNA coding for the vaccinating protein. Recent results obtained with infectious hematopoietic necrosis virus (Anderson et al. 1996) confirmed that genetic immunization can be effective with salmonids.

In our current funded research, the general immune status of spring chinook salmon fed different amounts of a controlled ration is being measured to investigate whether the levels of fat or the growth rate may be effecting their disease resistance, or the ability of fish to be vaccinated against BKD by genetic immunization. Understanding the relation between those factors and disease resistance in salmonids will be an immense benefit to all captive broodstock programs.

Element 3- Reintroduction strategies and reproductive success of captively-reared salmon

Improving reproductive performance of captively-reared adult fish

Recent studies conducted under previous funding periods have identified deficiencies in the reproductive success of captively reared coho and chinook salmon (e.g., Berejikian et al. 1997). The poor performance of captively reared salmon has been associated with differences in secondary sex characteristics (Hard et al. in prep), which develop in wild salmon as energy stores are metabolized during spawning migrations (Brett 1995). Poor fin quality and swimming performance have also been observed in captively reared chinook salmon released to spawn naturally in experimental channels and in natal streams. For adult reintroduction to be successful, culture environments must be developed that improve the morphology, physiology, and ultimately, reproductive performance of captively reared adult fish when released into their native habitat to spawn.

Exercise and starvation are natural parameters of anadromous salmonid migration, but in this respect, current husbandry practices do not simulate environmental conditions encountered by wild fish. Exercised fish are better adapted to exhaustive exercise, have greater red and white muscle mass, and exhibit reduced post-swimming stress acidosis than their non-exercised counterparts. Wild Atlantic salmon collected during their spawning migration exhibited extreme, persistent lactoacidosis, but no mortality after exhaustive exercise (Tufts, et al., 1991). Exercise and starvation increase red and white muscle mass and facilitate mobilization of stored reserves in juvenile steelhead (Barrett and McKeown, 1988). Exercise conditioning of striped bass (*Morone saxatilis*) increased growth, red and white muscle mass, and improved swimming performance (Young and Cech, 1994ab). Healing of wounded fins (fin wounding is very common in captive broodstocks) may also be improved by exercise conditioning (Joergensen and Jobling 1993). However, no studies have evaluated the effects of exercise conditioning on adult reproductive characters or performance.

We hypothesize that exercise conditioning of captively reared salmon through maturation will: 1) improve body composition (i.e, decrease fat and increase muscle mass), 2) promote greater development of secondary sex characteristics, and ultimately 3) improve reproductive performance. Improved female condition should increase their ability to dig nests, and deposit and cover eggs in the gravel. Improved development of male secondary sexual characteristics should increase female willingness (mate selection) to spawn with them (Berejikian et al. 1997).

Element 4- Quantitative Genetic Consequences of Captive Broodstock Programs

The primary focus of our present research to determine the genetic consequences of captive broodstock programs for natural salmon populations is a study of inbreeding

depression in chinook salmon. Inbreeding depression, a reduction in fitness caused by the mating of close relatives, has for decades been a prominent genetic concern of captive breeding programs involving threatened or endangered species. This concern stems from adverse effects of inbreeding on survival and reproductive capacity that have been well documented in many species of captively bred animals (Ralls and Ballou 1983), and experimental work has shown a strong link between the degree of inbreeding and fitness loss (Ralls et al. 1988). A recent study (Saccheri et al. 1998) has clearly demonstrated that reduced genetic variation associated with inbreeding can contribute directly to extinction of wild populations. Furthermore, evidence is mounting that a past history of inbreeding (e.g., due to historically small population size) does not necessarily buffer a population from subsequent inbreeding depression (Ballou 1997). The consequences of inbreeding in most salmonids are poorly understood; the relevant work has been limited to nonanadromous fish, especially brook and rainbow trout. Nevertheless, these studies found adverse effects of close inbreeding on survival and growth in these species (Hard and Hershberger 1995).

Even if inbreeding depression leads to higher risk of extinction, it is difficult to evaluate this risk relative to other risks, such as catastrophic loss or domestication of animals in captivity, and population fragmentation or local extinction in the wild. This is particularly true in light of recent evidence that inbreeding depression may reduce fitness sharply at intermediate levels of inbreeding (Frankham 1995) and its extent is likely to vary in different environments (Pray et al. 1994). Research on the consequences of inbreeding in anadromous salmonids would be most useful in characterizing the relationship between inbreeding and inbreeding depression and the environmental sensitivity of inbreeding depression. For captive broodstock programs, this information would help to evaluate the risk of inbreeding depression against other risks (such as the risk of domestication); this in turn would help to formulate guidelines for determining: 1) under what population scenarios a captive broodstock or captive rearing program should—and should not—be initiated based on current inbreeding levels, 2) what captive population sizes should be maintained and for how many generations, and 3) what characteristics of the captive environment are most important to simultaneously reduce risk of inbreeding depression and domestication.

b. Rationale and significance to Regional Programs

One of the current barriers to restoration of many depleted stocks of Pacific salmon (*Oncorhynchus* spp.) in the Columbia River Basin is the availability of suitable numbers of individuals for release back into their habitat. The Northwest Power Planning Council's Columbia River Basin Fish and Wildlife Program was recently amended to include development and implementation of captive broodstock technology to aid recovery of salmon stocks (Phase II; Measure VI.B.6.A.2). Captive broodstock programs are a form of artificial propagation that are intended to give listed stock a “jump start” on the road to recovery. Although captive broodstock programs for listed salmon are technologically well enough to be started, they are not without problems and risks to natural salmon populations. Captive broodstock programs can sustain high mortality, which may increase a population's risk of extinction if the captive component is a substantial fraction of this population. Rearing systems must be refined and operated to minimize the risk of loss due to disease, poor reproductive performance of adult fish, and poor survival of offspring once released into the native habitat. Additional risks include genetic change imposed on a population by a captive

broodstock program, genetic interaction between captive and natural fish in the wild, and ecological impacts of releases of captive fish on natural populations. The proposed project on technology assessment addresses some established problems and actively cooperates with ongoing captive broodstock programs to address new problems as they arise.

c. Relationships to other projects

Information from this project is used to improve technology used for ongoing Captive Broodstock Programs for Redfish Lake Sockeye Salmon and Snake River Chinook Salmon. Problems encountered by these programs are used to develop research needs for this research project.

d. Project history (for ongoing projects)

This project was initiated in 1993. During the first year an extensive literature review was conducted to assess the current status of captive broodstock technology and identify critical research needs. A multidisciplinary and multiagency research team was assembled to address identified research areas problems as they have emerged from ongoing captive broodstock programs. Research was initiated in FY 95. We are presently in our fourth year of research. Many of the ongoing experiments require rearing of fish for one or more generations, thus the major results of several studies initiated in FY95 will not be forthcoming until FY99. Major results and publications are summarized below.

Literature review:

Flagg, T.A., and C.V.W. Mahnken. 1995. An assessment of the status of captive broodstock technology for pacific salmon. Final report to the Bonneville Power Administration, Contract DE-AI79-93BP55064, 285 p. plus appendices (Available from Bonneville Power Administration, Public Information Center - CKPS-1, P.O. Box 3621, Portland, OR 97208).

Major Results:

1. Completed studies of effects of rearing sockeye salmon in fresh water or seawater on reproductive performance. Sockeye salmon broodstock reared in fresh water throughout the life cycle had higher survival than those reared for a period in seawater, but the timing of spawning, age of maturity and quality of gametes did not differ. However, body size of both sexes and egg size in seawater-reared fish were slightly smaller.

2. Completed studies on induction of ovulation and spermiation in sockeye salmon using gonadotropin-releasing hormone analog. Timing of spawning in mature sockeye salmon males and females can be controlled (advanced and synchronized) with gonadotropin-releasing hormone analog without impairing gamete quality. This technology is now available to hatchery managers to prevent loss of gametes due to prespawning mortality and synchronize male and female broodstock.

3. Completed studies on the effects of high and low growth rates, and varying levels of dietary fat on maturation of spring chinook salmon males. Both growth rate and body fat levels can affect the number of male spring chinook salmon maturing at 2 years of age, with growth rate being the predominant factor. The number of early maturing males was lowest when fish were reared on reduced ration and low fat/high protein diets. These results suggest that early maturation of male chinook salmon may be reduced by reducing growth rate.

4. Completed initial tests of azithromycin as a therapeutant for bacterial kidney disease in sockeye salmon. Azithromycin was more effective than erythromycin in reducing

mortality due to bacterial kidney disease. No apparent negative effect of the drug was found on fertilizability of eggs produced from broodstock treated with azithromycin.

5. Completed studies of the reproductive success of wild versus captive-reared coho salmon. Wild coho salmon spawned with captive-reared salmon, but wild males dominated captive-reared males resulting in fewer offspring from captive-reared males. There was no significant difference between the wild and hatchery-reared fish in the quality of gametes or survival of offspring. However, fry from wild females had more orange fin coloration than those from captive-reared females.

6. Completed initial study on the effects of feeding carotenoid-supplemented diets on reproductive performance of sockeye salmon broodstock. Carotenoid supplementation of broodstock diets did not alter gamete quality or the percentage of maturing fish.

7. Completed initial studies of the effects of live-foods on growth and behavior of first feeding fry. Fry fed commercial diets fed on live foods as well as those fed live-food from the time of first feeding. These results suggest behavioral imprinting on live food is not necessary for fry prior to release.

8. Completed development of standardized assays for cellular and humoral mediated immunity in sockeye salmon. Initial tests of immunocompetence of fish reared on either 8 or 12 C water indicated that no major differences in immune function could be detected.

9. Completed study on the effects of rearing temperature (8 or 12 C) on growth, development, smoltification, maturation and immune function of sockeye salmon. No major differences in immune function could be detected between the two groups. However, fish reared on 12 C were larger than those reared on 8 C. None of the fish reared on 8C matured at 3 years of age, compared to approximately 30% of those reared on 12C. The majority of fish matured at age 4 and there was no effect of rearing temperature on survival of offspring to the eyed-stage. These data indicate that rearing sockeye salmon at 12 C has no negative effect on gamete quality.

10. Completed initial phase of inbreeding experiment.

11. Successfully incorporated erythromycin into *Artemia* and a vehicle to treat first-feeding fry.

Captive Broodstock Research Project (9305600) Publications and Reports

Berejikian, B. A., E. P. Tezak, S. L. Schroder, C. M. Knudsen, and J. J. Hard. 1997.

Reproductive behavioral interactions between spawning wild and captive-reared coho salmon (*Oncorhynchus kisutch*). *ICES J. Mar. Sci.* 54:1040-1050.

Flagg, T. A., C. V. W. Mahnken, and K. A. Johnson. 1995. Captive broodstocks for recovery of depleted populations of Pacific salmon. *Am. Fish. Soc. Symp.* 15:81-90.

Flagg, T. A., and C. V. W. Mahnken (editors). 1995. An assessment of captive broodstock technology for Pacific salmon. Report to Bonneville Power Administration, Contract DE-AI79 93BP55064, 285 p. (Available from Northwest Fisheries Science Center, 2725 Montlake Blvd. E., Seattle, WA 98112). This report contains the following chapters:

- 1) The Captive Broodstock Concept: Application To Rearing Pacific Salmon, by T. A. Flagg, F. W. Waknitz and C. V. W. Mahnken, pp. 1-1 to 1-60.

- 2) Quantitative Genetic Consequences of Captive Broodstock Programs for Anadromous Pacific Salmon (*Oncorhynchus* spp.), by J. J. Hard and W.K. Hershberger, pp. 2-1 to 2-75.
- 3) Environmental and Endocrine Control of Reproduction in Cultured Salmonids, by P. Swanson, pp. 3-1 to 3-66.
- 4) Captive Salmon Broodstock Nutrition Literature Review, by I. P. Forster and R. W. Hardy, pp. 4-1 to 4-38.
- 5) Fish Health Aspects of Broodstock Restoration, by L. W. Harrell, pp. 5-1 to 5-14.
- 6) History of White River Spring Chinook Broodstocking and Captive Broodstock Rearing Efforts, by A. Appleby and K. Keown, pp. 6-1 to 6-32.

Hard, J. J. 1994. Genetics and salmon management: expanded summary of a panel discussion. In L. K. Park, P. Moran, and R. S. Waples (editors), *Applications of DNA technology to the management of Pacific salmon: proceedings of the workshop*, p. 151-163. U.S. Dep. Commer., NOAA Tech. Memo. NMFS-NWFSC-17.

Hard, J. J. 1995. Genetic monitoring of life-history characters in salmon supplementation: problems and opportunities. *Am. Fish. Soc. Symp.* 15:212-225.

Hard, J. J. 1995. A quantitative genetic perspective on the conservation of intraspecific diversity. *Am. Fish. Soc. Symp.* 17:304-326.

Kim, J., K.C. Massee, and R. W. Hardy. 1996. Adult *Artemia* as food for first feeding coho salmon (*Oncorhynchus kisutch*). *Aquaculture* 144:217-226.

Schiewe, M. H., T. A. Flagg, and B. A. Berejikian. 1997. The use of captive broodstocks for gene conservation of salmon in the western United States. *Bull. Natl. Res. Inst. Aquacult., Suppl.* 3:29-34.

Shearer, K. D., J. Silverstein and W. W. Dickhoff. 1997. Control of growth and adiposity in juvenile chinook salmon. *Aquaculture* 157:311-323.

Shearer, K. D., J. Silverstein, and E. M. Plisetskaya. 1997. The role of adiposity in food intake control of juvenile chinook salmon (*Oncorhynchus tshawytscha*). *Comp. Physiol. Biochem.* 118A:1209-1215.

Silverstein, J., K. D. Shearer, W. W. Dickhoff and E. M. Plisetskaya. In press. Regulation of nutrient intake and energy balance in salmon. *Aquaculture*.

Note that dates are not given for articles that have been accepted but not yet published by a journal. These would be cited in text as follows: Smith (In press).

Silverstein, J., K. D. Shearer, W. W. Dickhoff and E. M. Plisetskaya. In press. The roles of growth and fatness during a critical period in the sexual development of chinook salmon (*Oncorhynchus tshawytscha*). *Can. J. Fish. Aquat. Sci.*

Swanson, P., T. Flagg, J. J. Hard, L. Harrell, K. D. Shearer, R. Pascho, W. Hershberger, K. Massee, and R. Hardy. 1998. Research on Captive Broodstock Technology for Pacific Salmon. 1995 Report to Bonneville Power Administration, Contract DE-AI79 93BP55064. 181 p. (Available from Northwest Fisheries Science Center, 2725 Montlake Blvd. E., Seattle, WA 98112.). This report contains the following chapters:

- 1) Fish husbandry, by T. Flagg and C. McAuley, pp. 1-1 to 1-12.
- 2) Endocrine changes during maturation of Lake Wenatchee sockeye salmon (brood year 1990) reared in either fresh water or seawater, by P. Swanson, J. Dickey, A. Dittman, J. Athos, and A. Shafer, pp. 2-1 to 2-28.
- 3) Effects of rearing temperature on growth, reproductive performance, and immune function in captively reared sockeye salmon, by R. Pascho and P. Swanson, pp. 3-1 to 3-31.
- 4) Progress comparing the efficacy of azithromycin and erythromycin as therapeutic feed additives to control bacterial kidney disease in chinook salmon, by L. W. Harrell, pp. 4-1 to 4-12.
- 5) Captive broodstock nutrition research, by R. W. Hardy and K. Massee, pp. 5-1 to 5-18.
- 6) The effect of whole body lipid stores on early maturation of male spring chinook salmon (*Oncorhynchus tshawytscha*), by K. D. Shearer and P. Swanson, pp. 6-1 to 6-32.
- 7) Induction of ovulation and spermiation in sockeye salmon using gonadotropin-releasing hormone analog (GnRHa) in controlled-release devices, by P. Swanson, pp. 7-1 to 7-24.
- 8) Research on quantitative genetic consequences of captive broodstock programs for Pacific salmon populations, by J. J. Hard and W. K. Hershberger, pp. 8-1 to 8-24.

Captive Broodstock Project Manuscripts in Preparation or Submitted

Alcorn, S. and R. J. Pascho. In prep. Quantification of salmonid antigen-specific immunoglobulin by single-dilution enzyme-linked immunosorbent assay. (submitted to Fish Shellfish Immunol.)

Berejikian, B. A., E. P. Tezak, S. L. Schroder, and E. P. Beall. In prep. Male dominance and spawning behavior in wild and captively reared coho salmon (*Oncorhynchus kisutch*) under experimental conditions. (submitted to Trans. Am. Fish. Soc.)

- Berejikian, B. A., E. P. Tezak, S. L. Schroder, K. M. Knudsen, and T. A. Flagg. In prep. Competitive asymmetries between newly emerged offspring of captively reared and wild coho salmon (*Oncorhynchus kisutch*). (submitted to Trans. Am. Fish. Soc.)
- Hard, J. J., G. A. Winans, and J. C. Richardson. In prep. Phenotypic and genetic architecture of juvenile morphometry in chinook salmon. (submitted to J. Heredity)
- Hard, J. J., B. A. Berejikian, E. P. Tezak, S. L. Schroder, C. M. Knudsen, and L. T. Parker. In prep. Morphometric differentiation of wild and captively reared coho salmon (*Oncorhynchus kisutch*): a geometric analysis. (submitted to J. Fish Biol.)
- Hard, J. J., R. Pascho, J. Winton, D. Elliot, and L. Park. In prep. Heritability of infection in chinook salmon (*Oncorhynchus tshawytscha*) by *Renibacterium salmoninarum*. (submitted to Trans. Am. Fish. Soc.)
- Hardy, R. W., K. C. Massee, and C. Rathbone. In prep. Growth, feed efficiency and maturation in sockeye salmon fed diets differing in lipid content at two feeding levels.
- Hardy, R. W. and K. C. Massee. In prep. Growth, survival, and reproductive performance of sockeye salmon fed a nutritionally-enhanced diet.
- Massee, K. C., J. Kim, B. A. Berejikian, and R. W. Hardy. In prep. Prey selection of naive and experienced juvenile sockeye salmon.
- Shearer, K. D. and P. Swanson. In prep. The effect of whole body lipid on early sexual maturation of 1+ age male chinook salmon (*Oncorhynchus tshawytscha*). (submitted to Aquaculture)
- Swanson, P., D. Mylonas, D. Larsen, W. W. Dickhoff, and Y. Zohar. In prep. Induction of spawning in Pacific salmon using gonadotropin-releasing hormone analogues in biodegradable or non-biodegradable controlled-release devices. (submitted to Aquaculture)
- Swanson, P., B. Berejikian, T. Flagg, J. J. Hard, L. Harrell, K. D. Shearer, R. Pascho, W. Hershberger, K. Massee, and R. Hardy. Research on Captive Broodstock Technology for Pacific Salmon. 1996 Report to Bonneville Power Administration, Contract DE-AI79 93BP55064. 181 p. (Available from Northwest Fisheries Science Center, 2725 Montlake Blvd. E., Seattle, WA 98112.). (In NMFS editorial review)
- Swanson, P., B. Berejikian, T. Flagg, J. J. Hard, L. Harrell, K. D. Shearer, R. Pascho, W. Hershberger, K. Massee, and R. Hardy. Research on Captive Broodstock Technology for Pacific Salmon. 1997 Report to Bonneville Power Administration, Contract DE-AI79 93BP55064. 181 p. (Available from Northwest Fisheries Science Center, 2725 Montlake Blvd. E., Seattle, WA 98112.). (In NMFS editorial review)

- Berejikian, B. 1997. Reproductive behavioral interactions between spawning wild and captively reared coho salmon. ICES/NASCO symposium: Interactions between hatchery and wild populations of Atlantic salmon, Bath, UK.
- Berejikian, B. 1997. Comparisons of reproductive ecology of captive and wild coho salmon. Columbia Basin Fish and Wildlife Authority Project Review, Portland, OR.
- Berejikian, B. 1998. Studies on the reproductive success of captively reared and wild coho salmon: a cooperative project between the National Marine Fisheries Service, Long Live the Kings, and the Hood Canal Salmon Enhancement Group. Hood Canal Salmon Enhancement Group Annual Meeting, Belfair, WA.
- Berejikian, B. A., E. P. Tezak, S. L. Schroder, C. M. Knudsen, J. J. Hard, and L. Park. March 18-20, 1998. Reproductive behavioral interactions between wild and captively reared coho salmon. Paper presented at the American Fisheries Society Annual General Meeting, Union, WA.
- Flagg, T. 1993. Redfish Lake sockeye salmon broodstock programs. Alaska Department of Fish and Game Sockeye Salmon Workshop, Cooper Landing, AK.
- Flagg, T. 1994. Captive broodstocks for recovery of depleted populations of Pacific salmon. American Fisheries Society Symposium on the Uses and Effects of Cultured Fishes in Aquatic Ecosystems, Albuquerque, NM.
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- Hard, J. J. 1994. A quantitative genetic perspective on the conservation of intraspecific diversity. American Fisheries Society Symposium on Evolution and the Aquatic Ecosystem, Monterey, CA.
- Hard, J. J. December 1994. Artificial propagation of Pacific salmon under the Endangered Species Act: constraints and opportunities. Northwest Fish Culture Conference, Sunriver, OR.
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Committee Participation

Stanley Basin Sockeye Technical Oversight Committee: Tom Flagg, Carlin McAuley.

Chinook Salmon Captive Propagation Technical Oversight Committee: Tom Flagg, Carlin McAuley.

Chinook Salmon Captive Broodstock Technical Oversight Team, Oregon: Carlin McAuley.

e. Proposal objectives

Element 1- Effects of Growth and Diet on Age of Maturity, Smoltification, Body Coloration, and Gamete Quality in Chinook and Sockeye Salmon

Objective 1.1 The effect of growth rate on early male maturation, smoltification and immunocompetence in juvenile spring chinook salmon containing low levels of body fat.

Objective 1.2 Determine effects of reduced temperature and ration during winter months on early male maturation in chinook salmon

Objective 1.3 Determine effects of constant vs. variable dietary protein and energy intake on growth, body conformation, natural spawning success, spawning behavior, and reproductive performance of chinook salmon.

Element 2- Fish Health

Objective 2.1 Monitor the nonspecific and specific immune functions of groups of spring chinook salmon reared on high protein low fat diets and reduced water temperature during winter months (Objective 1.2).

Objective 2.2: Test the effectiveness of azithromycin as an emergency treatment against acute BKD in chinook salmon (*Oncorhynchus tshawytscha*).

Objective 2.3 Effects of long-term anti-BKD prophylactic treatment regimens with erythromycin on disease incidence, gonad development, and gamete quality in fall chinook salmon (*Oncorhynchus tshawytscha*).

Element 3- Reintroduction strategies and reproductive success of captive-reared salmon

Objective 3.1 Determine the effects of exercise on body composition, morphology and breeding success of captive reared chinook salmon.

Objective 3.2 Determine the relative effects of natural emergence, remote site incubation (egg boxes), and fry releases on the early growth of chinook salmon juveniles.

Objective 3.3 Compare the natural breeding success of freshwater- and sea water-reared chinook salmon in their natal habitat

Element 4- Quantitative Genetic Consequences of Captive Broodstock Programs

Objective 4.1 Determine effects of inbreeding depression on fitness of a chinook salmon population

f. Methods

Element 1- Effects of Growth and Diet on Age of Maturity, Smoltification, Body Coloration, and Gamete Quality in Chinook and Sockeye Salmon

Objective 1.1 The effect of growth rate on early male maturation, smoltification and immunocompetence in juvenile spring chinook salmon containing low levels of body fat.

In spring 1998, we initiated an experiment to determine the effects of growth rate on survival, growth rate, smoltification, age of maturity, immunocompetence, adult body size, fecundity and egg size. Six groups (in duplicate) of Willamette River spring chinook salmon (1997 brood) are being reared from start feeding to age 2+ on graded rations (100= satiation, 90, 80 70 60 and 50% of satiation) of a high protein (55%), low fat (8%) feed (Table 1) and a seventh group is being fed BioDiet at the 60% level, which serves as a commercial diet control group. Two additional replicate tanks of the 100% ration were added to permit extensive sampling of maturing and nonmaturing fish within one treatment group.

Table 1. Composition of experimental diets
(based on estimated proximate composition of feed

ingredients).

<u>Ingredient</u>	<u>(g/kg)</u>
Anchovy meal -herring meal mix (1:)	550
Concentrated liquid fish	200
Wheat midts	140
Whey	20
Vitamin premix	30
Vitamin C	10
Trace element premix	10
Krill meal	30
Choline chloride	10
CMC	5
Guar gum	5

Each tank initially contained 700 fish. Fish are being reared in recirculated fresh water at the fish holding facilities at the Northwest Fisheries Science Center, Seattle, WA. Water temperatures fluctuate between 10-12° C. Artificial light on a natural photoperiod is being used. Fish in each tank are being batch weighed and counted monthly to determine the mean fish weight in each tank. Twenty fish are being individually weighed and measured monthly to determine condition factor. Samples for proximate analysis and visual examination of testes are being collected every month (10 fish/tank). To monitor the effects of the treatments on smoltification, samples of gill tissue for gill Na/K ATPase and blood plasma for thyroxine and insulin-like growth factor analyses are being collected from 6 fish per tank during Oct-Dec of 1998 and Mar-May and Oct-Dec of 1999. For the monitoring of smoltification samples will be collected biweekly during the aforementioned periods. Immunocompetence will be assessed by Northwest Biological Research Division (USGS) personnel using methods described in Objective 2.2. Briefly, sixty fish from each tank are being challenged at three points in the study (Table 2). To monitor effects of the treatment groups on maturation, gonads are removed and weighed in all fish sacrificed for sample collection. In addition, we sample fish from the 100% ration groups for which we have two additional replicate tanks. From June 1998 through October 1999 we are sampling 10-20 fish per tank with the goal of obtaining sufficient numbers of maturing and nonmaturing male fish. Samples of pituitary glands and blood are being collected for gonadotropin analysis, and gonad tissue will be collected for histology. Results of this analysis will aid us in determining more precisely critical periods for initiation of sexual maturation in this species.

The final sampling will take place in October 1999. At this time all fish will be sexed and the incidence of male maturation will be assessed by determining gonadosomatic indices of all fish. If facilities are available, groups of fish from each treatment will be maintained for another 2 years (through October 200) to evaluate effects of ration on female age of maturity, fecundity, and egg size. We are concerned that reduced ration may be advantageous to reduce maturation of 2-year old males, but may have negative consequences on female maturation in the subsequent years.

Table 2. Sampling plan each year and month.

	<u>1998</u>	<u>1999</u>
ND	JFMAMJASOND	JFMAMJJASO
1234		
GGGGGGGGGG	GGGGGGGGG	
	III	IIII
	PPPPPPPPPP	PPPPPPPPPP

1 obtain eggs, 2 first feeding, 3 distribute fish in to tank sand begin variable ration treatments, 4 final sampling and terminate experiment

I= immunocompetence challenge

P= proximate composition determination

S= smoltification status determination

G= samples for gonad histology and gonadotropin measurements

The effect of ration on body composition will be determined using allometric analysis of body weight versus total body fat. The effect of growth rate on smoltification and immunocompetence will be determined by regression. The relationship between growth rate and the incidence of sexual maturation (%) will be examined using regression after arcsin transformation of percentage data. Sample and data analysis will be done during October 1999 through October 2000.

Objective 1.2 Determine effects of reduced temperature and ration during winter months on early male maturation in chinook salmon

As of autumn 1998, the growth of fish reared in the experiment described in Objective 1.1 has far exceeded that of wild or typical hatchery-reared fish, yet fish have body fat levels of less than 4%, which is about half that of a typical hatchery reared fish. To date, no significant maturation at one year of age was observed in the experimental groups despite the large size of yearling fish (60-100 g body weight). In an attempt to mimic a more natural growth cycle, we propose to test whether fish fed a high protein low fat diet and exposed to a combination of reduced temperature and ration during the winter months exhibit a seasonal growth cycle more similar to wild fish. We will evaluate the effects of treatments on growth, smoltification, age of maturity, and disease resistance. Willamette River spring chinook salmon (broodyear 1998) will be fed at first feeding the diet described in Table 1. Fish will be divided into four groups (duplicate tanks per group): 1) high ration , constant temperature; 2) high ration, reduced winter temperature; 3) low ration, constant temperature; and 4) low ration, reduced winter temperature. The level of ration will be determined from results of experiment described in Objective 1.1. Fish will be reared in recirculated fresh water at the fish holding facilities at the Northwest Fisheries Science Center, Seattle, WA. Water temperatures in the constant temperature group will range from 10-12° C. In the reduced winter temperature groups, water temperature during November through February will be reduced to 6 C. Artificial light on a natural photoperiod is being used. Fish in each tank are being batch weighed and counted monthly to determine the mean fish weight in each tank. Twenty fish are being individually weighed and measured monthly to determine condition

factor. Samples for proximate analysis and visual examination of testes are being collected every month (10 fish/tank). To monitor the effects of the treatments on smoltification, samples of gill tissue for gill Na/K ATPase and blood plasma for thyroxine and insulin-like growth factor analyses are being collected from 6 fish per tank during Oct-Dec of 1999 and Mar-May and Oct-Dec of 2000. For the monitoring of smoltification samples will be collected biweekly during the aforementioned periods. Immunocompetence will be assessed by Northwest Biological Research Division (USGS) personnel using methods described in Objective 2.2. To monitor effects of the treatment groups on maturation, gonads are removed and weighed in all fish sacrificed for sample collection. The final sampling will be during Dec. 2000 when the reproductive status of all fish will be assessed. The effects of treatment on growth, percentage maturing fish, smoltification and immunocompetence will be determined by ANOVA.

Objective 1.3 Determine effects of constant vs. variable dietary protein and energy intake on growth, body conformation, natural spawning success, spawning behavior, and reproductive performance of chinook salmon.

Chinook salmon of the Priest Rapids strain (1998 brood year) will be obtained as eggs from the Washington Department of Fish and Game Sandpoint and transferred to the fish culture laboratory of the Aquaculture Research Institute, University of Idaho, Moscow, Idaho. The fish will be divided into four dietary treatment groups, with two replicate groups receiving each dietary treatment. Subsamples of juvenile fish from two dietary treatments will be evaluated with respect to behavior, coloration, and fitness in connection with juvenile re-introduction strategies. The majority of the fish will continue in these dietary treatment groups to adult maturation. These fish and fish from the other two dietary treatment groups will mature in Fall, 2001 or 2002, meaning that they will be exposed to the various dietary treatments for three or four years before spawning. The fish will be bulk-weighed, measured for length, and counted quarterly, and two fish from each replicate tank will be removed quarterly for determination of gonadosomatic index (GSI), and proximate composition. At adult maturation, males and females will be identified, and fish from each dietary treatment group will be split into two groups, one to continue in tanks until spawning, and the other to be placed in an observation channel (artificial stream) for behavioral studies to assess their ability to compete with wild maturing chinook salmon and successfully spawn. The wild maturing chinook salmon will be the cohorts of our captively-reared fish, captured as they return to Priest Rapids. Thus, this study will provide an opportunity to produce fish of differing size, conformation, and external coloration and test their reproductive success in an experimental setting similar to that in the wild. This portion of our proposed research will be conducted in concert with similar studies at the Manchester Experimental Laboratory, NMFS, Manchester, Washington, and be designed to complement on-going behavioral research at that facility. Fish that remain in tanks will be spawned in a factorial design so that eggs from each female are fertilized with milt from three males from each dietary treatment, and vice versa. A total of 20-25 males and females from each dietary treatment group will be spawned, and survival of offspring from each cross to swim-up will be enumerated.

Element 2- Fish Health

Objective 2.1 Monitor the nonspecific and specific immune functions of groups of spring chinook salmon reared on high protein low fat diets and reduced water temperature during winter months (Objective 1.2).

During 2000 and 2001, the immunological competence of fish in each group will be assessed once by measuring selected factors related to specific and nonspecific host defense mechanisms (Swanson et al. 1995). The results from these measurements will provide relative estimates of the general immune status of fish from each of the groups, and if the levels of a given diet component affect their disease resistance. Fish for this experiment will be reared as described in Objective 1.2. We will test the following null hypothesis: There will be no detectable differences in the humoral responses to the p57 protein of *R. salmoninarum* by groups of spring chinook salmon fed a modified commercial diet with various levels of one component.

To measure the specific immune response to the p57 protein of *R. salmoninarum*, a recombinant p57 protein will be produced in an *E. coli* expression system as previously described, and used to vaccinate fish from each ration group. At specific intervals following vaccination, fish from each vaccinate group will be bled and their specific humoral response to the p57 protein will be measured with the IgM-ELISA. The relative abilities of fish from four treatment groups to produce a humoral response to the recombinant p57 protein will be compared during early summer (Mid-June) of 1999, and early spring (March-May) and early summer (May -July) of 2000.

Experimental subgroups and rations. For each vaccination trial, 60 fish will be removed from each of the two replicate tanks in the appropriate ration groups. After vaccination, the fish in those subgroups will be maintained on the same feed ration as the tank from which they originated

Vaccination with the p57 protein. All of the fish in each subgroup will be injected intraperitoneally with 200 μ L of a single concentration of the recombinant p57 protein in Freund's incomplete adjuvant. Following vaccination, subgroups will be reared separately under the same conditions as the corresponding experimental (ration) groups.

The levels of specific serum immunoglobulin to the p57 protein will be measured in unvaccinated fish, and in fish sacrificed at regular intervals after vaccination. To obtain serum from unvaccinated fish, individual blood samples will be taken from 10 fish sacrificed from each subgroup just before injection of the p57 protein. Each blood sample will be clotted overnight at 4°C, then centrifuged at 2,000 x g for 10 min to recover a serum sample for analysis in the IgM-ELISA. The levels of specific immunoglobulin among diet groups after vaccination will be compared by analysis of variance ($p \leq 0.05$). Sample sizes are based on the criteria described by Amend 1981.

We will also test the following null hypothesis:

There will be no detectable differences among treatment groups in the humoral responses to a DNA vaccine based on the gene for the p57 protein of *R. salmoninarum* various levels of one component.

The ability of fish from each of the diet groups to produce a humoral response to the p57 protein of *R. salmoninarum* after vaccination with a DNA vaccine will be evaluated during the spring (April-June) of 1999. The methods will be based on those described by Anderson et al. (1996). Control bacterins will include killed *R. salmoninarum* and the p57 protein in complete

Freund's adjuvant. Monitoring of the humoral response and quantification of immunoglobulin levels will be done as described above. All vaccinations will be done at the Western Fisheries Research Center, Seattle, Washington. Statistics. The levels of specific immunoglobulin among diet groups after vaccination will be compared by analysis of variance ($p \leq 0.05$). Sample sizes are based on the criteria described by Amend 1981.

Finally, we will test the following null hypothesis: There will be no detectable differences in the nonspecific immune functions among groups of spring chinook salmon fed a modified commercial diet with various levels of one component.

The nonspecific immune functions of fish from the ration groups will be measured during August 1999 and June-July 2000 according to the methods described by Swanson et al. (1995). For each evaluation, subgroups of 30 fish will be randomly chosen from each feed group. Briefly, a total of 15 fish will be tested from each replicate tank of a given feed group; five fish will be removed from each tank by repeated dip-netting every other day for 5 days. Fish will be anesthetized with tricaine methanesulfonate (MS-222), then the weight and fork length was determined to calculate the condition factor. The condition factor will be calculated according to the method described by Piper et al. (1982). Condition factor values will be corrected for the use of metric measurements by the formula $C = 36.14K$, where C and K are condition factors based on English and metric units, respectively. A blood sample will be taken by caudal vein puncture for the hematocrit, leukocrit, and plasma protein determinations. Duplicate smears of whole blood will be made for each fish, then the remainder of each sample was allowed to clot overnight at 4°C and centrifuged at 5,000 x g for 20 min. The serum divided into 2 vials and stored at -80°C.

On each sample date, a five fish-pool of anterior kidney tissue will be prepared from each subgroup. The anterior kidney will be aseptically removed and a small portion from the center of the sample used to make kidney several imprints on each of two glass microscope slides. The tissue imprints will be air-dried, then fixed in absolute methanol for 5 minutes for differential cell counts. The other slide will be stored at 4°C for the myeloperoxidase assay. Leucocytes will be purified from the remaining tissue in each pool by gradient centrifugation for use in the phagocytosis and NBT assays. A single factor nested ANOVA ($P \leq 0.05$) will be used to compare means among the temperature groups for certain nonspecific immune parameters. Count data will be compared by chi-square analysis ($P \leq 0.05$). Sample sizes are based on the criteria established by Amend 1981.

Objective 2.2: Test the effectiveness of azithromycin as an emergency treatment against acute BKD in chinook salmon (*Oncorhynchus tshawytscha*).

Results obtained in our previous studies with sockeye salmon demonstrate that azithromycin can be extremely useful in preventing BKD from overwhelming a captive broodstock. However, routine use of azithromycin or any antibiotic to prophylactically treat bacterial disease in animals should be avoided when possible to reduce the chance of antibiotic resistance development. A recent report from the World Health Organization entitled "The Medical Impact of the Use of Antimicrobials in Food Animals" recommends prudent use of antibiotics where supported by appropriate diagnostic tests. While this report was directed at the use of antimicrobials in food animals, it is sound advice for the use of

antimicrobials in all situations. Acquisition of antimicrobial resistance by bacteria is increasingly commonplace, and in fact, azithromycin resistant bacteria have already developed during treatment of human infections (Bermudez et al. 1998; Hoge et al. 1998). Couple this with the expense of this antibiotic and the fact that it will probably never be approved for routine prophylactic use in fish, it is clear that the use of azithromycin or other antimicrobials should be viewed as a “last resort” option for endangered populations. The following experimental plan takes this view into account. It should also be pointed out that while evidence acquired in our preliminary studies suggests that higher survival rates are obtained in BKD-challenged sockeye salmon treated with azithromycin versus erythromycin, a limited analysis of these survivors for the presence of *R. salmoninarum* by the sensitive RT-PCR assay shows that at least some of the fish are still chronically infected, showing evidence of the bacterium in blood and ovarian fluid. Therefore it will be important to determine if the progeny of these survivors are also infected, in order to assist the decision making of captive broodstock managers who must evaluate whether it is appropriate and safe to return these fish to their native habitat.

Experiments carried out in fiscal years 1998 and 1999 have focused on comparisons of erythromycin vs. azithromycin as therapeutic agents in chinook salmon previously infected with *R. salmoninarum* and showing pathological signs of acute BKD, in order to mimic a more realistic scenario of disease presentation. These experiments will be extended to determine optimum dosage levels and the effects of multiple treatments on appearance of overt clinical disease. Fish experimentally infected and treated in this manner will be analyzed for the presence of *R. salmoninarum* by our most sensitive methods (RT-PCR) in order to determine whether complete clearance of the organism is attainable. General experimental protocols previously developed and described will be followed.

Approximately 1400 fall chinook or sockeye salmon will be separated into two equal groups in tanks supplied with pathogen free seawater at the Manchester Laboratory; 1 group will be designated as unchallenged fish, and one designated as BKD-challenged. Fish will be fed a standard unmedicated diet, and during adaptation to the tanks, randomly selected fish will be screened for the presence of BKD by ELISA and FAT of kidney tissues, and by RT-PCR of blood samples. Prior year pre-screening has shown that ~25% of the fish infected with *R. salmoninarum*. The group designated for challenge will be inoculated intraperitoneally (IP) with a dose of *R. salmoninarum* (ATCC strain 33209) designed to produce clinical acute BKD (approximately 1×10^6 bacteria/12 g fish). The group designated unchallenged will be inoculated IP with an equivalent volume of phosphate buffered saline to duplicate the stress of handling and inoculation of the challenged fish. This group will also serve as a negative control for any deleterious effects of subsequent antibiotic treatment, activation of quiescent pre-existing BKD, or the appearance of other unrelated disease and mortality.

After inoculation, the fish will be randomized and transferred into 8 4-foot diameter tanks per group, with each tank containing 75 fish. Ten days post challenge (a time period preliminary experiments showed that first mortalities from this challenge would occur), fish will be switched to feed supplemented with the antimicrobials (duplicate tanks for each). Initial experiments have used erythromycin at 100mg/Kg/fish/day for 28 days (standard dosage under University of Idaho INAD), azithromycin at 10 mg/Kg/fish/day for 14 days (low dose), azithromycin at 30 mg/Kg/fish/day for 14 days (high dose), and no medication.

Followup experiments will include a broader range of azithromycin dosages (5-50 mg/Kg/fish/day), and multiple treatments from 2-4 times in a year. The health of the fish will be monitored, and all mortalities necropsied to verify death by BKD. Kidney tissues will be examined by FAT to determine levels of *R. salmoninarum*. Representative fish from all treatments will be routinely sampled and analyzed for the presence of the bacterium. In older fish where blood draws are feasible, the RT-PCR assay for *R. salmoninarum* will be performed on blood in order not to sacrifice the fish.

Representative survivors as well as the uninfected control fish will be maintained in captivity to determine the effect of azithromycin treatment on sexual maturation, egg quality, and reproductive success, in conjunction with Objective 2.3. These experiments will capitalize on the effort expended to initiate this subtask and will confirm the results observed previously in our studies of azithromycin in sockeye salmon.

Objective 2.3 Effects of long-term anti-BKD prophylactic treatment regimens with erythromycin on disease incidence, gonad development, and gamete quality in fall chinook salmon (*Oncorhynchus tshawytscha*).

There are three main goals under this objective. The first is to determine how captive fall chinook salmon broodstock gonad development, gamete viability, and survival of the progeny through the swim-up stage is affected by long-term prophylactic administration of erythromycin or azithromycin. Secondly, if the experimental fish are found to be naturally infected with *R. salmoninarum*, we will measure the effects of two erythromycin treatment regimens (two or four treatments annually) on BKD incidence, from the first feeding fry through mature adult life history stages. And lastly, this study will allow us to determine whether erythromycin toxicity responses are related to treatment frequency; this aspect of the study will include a measurement of residual tissue concentrations and will document underlying tissue damage associated with the syndrome. Initial experiments will focus on erythromycin as it is the current antibiotic of choice and the one apparently causing the severe toxic side effects. In the following year(s) similar studies will examine the long-term effects of azithromycin treatment, especially if experiments carried out in Objective 2.2 continue to show that this antibiotic is more efficacious for treatment of BKD in a captive broodstock setting.

The initial first year experimental plan will include the following treatments:

Phase 1 – First feeding to smolt stage

1. No treatment.
2. Erythromycin administered orally at a rate of 100 mg/kg fish body weight per day for 28 days, with the first treatment administered at initiation of exogenous feeding, the second treatment administered during sexual differentiation (2 g average weight), and the third just prior to smoltification (ca. 7 g average weight).
3. Erythromycin administered orally at a rate of 100 mg/kg fish body weight per day for 28 days, with the first treatment administered during sexual differentiation (2 g average weight), and the second just prior to smoltification (ca. 7 g average weight).
4. Erythromycin administered orally at a rate of 100 mg/kg fish body weight per day for 28 days, with the only treatment administered just prior to smoltification (ca. 7 g average weight).

Phase 2 – Smolt to mature adult stage

1. Erythromycin administered orally at a rate of 100 mg/kg fish body weight per day for 28 days, with one treatment every 90 days beginning at trial initiation and continuing until 3 months prior to spawning.
2. Erythromycin administered orally at a rate of 100 mg/kg fish body weight per day for 28 days, with one treatment every 180 days beginning at trial initiation and continuing until 3 months prior to spawning.
3. No treatment.

The following experimental plan will be followed. George Adams Fall Chinook Salmon will be transferred to the Big Beef Creek Hatchery. During the fry to smolt stage the fish will be held indoors in fourteen 2 m x 1 m circular tanks. During the smolt to mature adult stage the fish will be held outdoors in six 6 m x 1.5 m circular tanks. Tanks, equipment, and fish used in the study will be physically isolated from ESA stocks. Just prior to initiation of exogenous feeding, 400 fish will be randomly stocked into each of 14 isolation tanks (two tanks per experimental treatment). At smoltification, 120 fish from each tank will be PIT tagged and divided randomly into three groups of 40 fish each which will be combined as follows:

- Tank 1: Forty fish from Treatments 1-7, replicate A
- Tank 2: Forty fish from Treatments 1-7, replicate A
- Tank 3: Forty fish from Treatments 1-7, replicate A
- Tank 4: Forty fish from Treatments 1-7, replicate B
- Tank 5: Forty fish from Treatments 1-7, replicate B
- Tank 6: Forty fish from Treatments 1-7, replicate B

Feeding procedures and growth management: First feeding fish will be fed with artemia for at least 28 days, until the fish receiving medication complete their course of treatment. Thereafter, fish will be fed using timer activated automatic feeders with supplemental hand feeding of the unmedicated diets, according to predetermined growth schedules which have been developed from existing data. Feeding levels will be adjusted to produce similar growth in all experimental treatments. Fish will be fed by hand during all periods of medication.

Data collection: Fish in each treatment will be weighed and measured at monthly intervals, during the presmolt stage, and once per year just prior to spawning (October). During the presmolt stage, fish will be collected before the initiation of exogenous feeding, and after each antibiotic treatment regimen. After smoltification, sampling will be conducted once annually, coincident with the onset of final maturation. At least ten female and ten male fish will be collected from each replicate. Histological examination of the gonads to determine initiation and extent of tissue damage, and for residual erythromycin levels. Liver, kidney, spleen, and brain tissues will also be examined for signs of erythromycin toxicity. Kidney tissues collected at each scheduled sampling, or obtained from moribund or dead fish will be examined for the presence of *Renibacterium salmoninarum* using the fluorescent antibody (DFAT) and/or RT-PCR techniques.

Gamete quality: Females from each treatment group will be paired with males in a factorial arrangement which will permit detailed evaluation of male and female gamete quality. Fertilized eggs from each mating will be incubated in isolation to hatching. Subsamples of eggs will be examined to measure fertilization and survival to the eyed stage. Fry will be counted, measured, and weighed at swim-up, and samples taken for BKD analysis.

Element 3- Reintroduction strategies and reproductive success of captive-reared salmon

Objective 3.1 Determine the effects of exercise on body composition, morphology and breeding success of captive reared chinook salmon.

Offspring of wild Dungeness spring chinook salmon (1996 brood year) will be cultured under two different rearing regimes at the NMFS Manchester Marine Experimental Station. Two hundred, fifty-four chinook salmon, will be divided into four 13 ft circular tanks supplied with pathogen-free sea water. Fish in two of the tanks will be cultured under existing protocols for endangered chinook salmon populations. Fish in the other two tanks will be subjected to increased current velocities in the tanks (greater than 1.0 body length per second; Berg 1996) for several hours each day.

The morphology of sexually mature chinook salmon grown in high and low velocity tanks will be compared by measuring morphometric characters associated with sexual maturation and reproductive fitness (kype length, snout-eye distance, girth, body depth, head shape, caudal peduncle depth, and caudal fin area). Each character will be adjusted for body size by regressing each character against body weight and calculating residual values from that relationship (Fleming and Gross 1994). Residual character values will be analyzed by multivariate ANOVA to determine whether exercise affected the morphology of these adults. The body composition of chinook salmon adults grown under the two treatments will be analyzed for protein and fat levels. The findings will be compared to similar analyses of wild chinook salmon obtained from published literature.

Samples for morphological and body composition analyses will include all the fish used in the spawning experiment (sampled at death), as well as all remaining fish not used in the spawning experiment.

To assess reproductive performance, all four possible mating combinations of exercised (E) and non-exercised (NE) males and females (i.e., E (with E (, E (with NE (, NE (with E (, NE (with E () will be created, and male-female pairs will be placed into an experimental stream channel at Manchester. Eight pairs for each combination will be placed into isolated 5-m long by 3-m wide stream channel sections (one pair per section). The frequency of courtship, nest digging and aggressive behaviors exhibited by each pair will be recorded by repeated scan sampling from the time they are introduced until spawning is complete. During periods of nest construction and spawning, behavior will be recorded on video tape by placing remote video cameras into the appropriate sections. Continuous pre-and post-spawning behavior will be collected in this fashion for as many spawning events as possible. The scan and focal (video) sampling will provide for quantification of: 1) total spawning duration (time from first to last spawning), 2) number of spawning events, 3) pre-spawning courtship, nest digging and aggressive behavior, and 4) post-spawning behavior. Percent egg deposition (an important measure of female breeding success) will be calculated by subtracting the number of eggs retained at death from the female's estimated fecundity. Statistical analyses of each dependent variable will be conducted using a two-way ANCOVA

with female population, male population and their interaction as the main effects and female body weight as the covariate.

We also propose under this objective to establish and monitor an exercise rearing program for a steelhead (*O. mykiss*) captive broodstock (1998 brood year). Reproductive success experiments will be conducted on mature age-4 adults in 2002 to test null hypotheses similar to those stated above.

Objective 3.2 Determine the relative effects of natural emergence, remote site incubation (egg boxes), and fry releases on the early growth of chinook salmon juveniles.

In addition to releasing ESA-listed captively reared chinook salmon adults for natural spawning, some artificially spawned embryos were stocked into remote site incubation (RSI) systems in several Salmon River, Idaho tributaries in 1998. Evaluation of RSI success in these programs is problematic because no external marks can be applied and identification of internal marks (e.g., thermal otolith marks) requires lethal sampling. Thus, we propose experimental studies using a surrogate, non-listed population.

We will test the following null hypothesis: Growth of fry from three possible release strategies (eggs buried in gravel, eggs stocked in stream-side incubators, and emergent fry release) does not differ either in the presence or absence of competition with fish produced from the other release strategies. At the eyed stage of development, eggs from a common lot will be allocated to one of the three release strategies in the experimental stream channel at the NMFS Manchester facility. The stream channel will be divided into 16 sections that are similar in area, flow, depth, velocity, substrate, and insect production. Eyed eggs will either be 1) placed in hatch boxes and buried in artificially created nests, 2) placed into a remote site incubator (RSI), or 3) stocked into the channel as newly emerged fry. Four of the sections will receive fish from an RSI (one RSI per section), four will receive buried eggs, four will receive fry from hatchery incubators, and four will receive eggs or fry from each of the three strategies (to examine competitive effects on growth). Daily instantaneous growth rates of fish from each strategy will be analyzed by a ANOVA.

Objective 3.3 Compare the natural breeding success of freshwater- and sea water-reared chinook salmon in their natal habitat

The first releases of ESA-listed adult captively reared chinook salmon occurred in 1998. Preliminary information from the Idaho Department of Fish and Game (P. Hassemer, pers. comm.) indicates that female chinook salmon reared in sea water (NMFS, Manchester, WA) may have exhibited greater spawning success than those reared in freshwater (IDFG, Nampa, ID). In future years, captively reared adults will continue to be released into a section of Bear Valley Creek, Idaho, where they can be observed from the stream banks. Access of adult salmon in and out of the stream section can be controlled at a temporary weir.

We will test the following null hypothesis: breeding success of captively reared chinook salmon does not depend on whether they were reared in fresh or sea water. In cooperation with the IDFG Monitoring and Evaluation Program, behavioral measures of breeding success and estimates of egg deposition will be collected on adults in a similar manner as that described in Objective 3.1. Because these fish will have the ability to move throughout the

stream section (several kilometers), their movements will also be tracked, and locations recorded using a Global Positioning System. Data on breeding behavior and success of both groups will be analyzed separately for each sex by ANCOVA.

Element 4- Quantitative Genetic Consequences of Captive Broodstock Programs

Objective 4.1 Inbreeding depression

The objective of the current study is to determine the effects of controlled inbreeding on survival, development, age structure, and other aspects of the life history of chinook salmon. We are testing three basic hypotheses:

H01: Inbreeding depression does not reduce viability or alter life history characteristics of chinook salmon.

Ha11: Inbreeding depression reduces viability during early life history but does not affect development rate, age structure, or reproductive capacity.

Ha12: Inbreeding depression has effects throughout the life cycle.

H02: The degree of inbreeding has no predictable effect on inbreeding depression in chinook salmon.

Ha21: The relationship between inbreeding and inbreeding depression is linear.

Ha22: The relationship between inbreeding and inbreeding depression is nonlinear.

H03: Inbreeding depression in chinook salmon does not vary between captive (i.e., protective culture throughout life cycle) and hatchery (i.e., protective culture from embryo to smolt) environments.

Ha31: Inbreeding depression is greater in a hatchery than in a captive environment.

Ha32: Inbreeding depression is greater in a captive than in a hatchery environment.

As of autumn 1998, the study is at the beginning of its fifth year. To date we have 1) established, using a conventional quantitative genetic breeding design, an experimental hatchery population of 1994-brood Puget Sound fall chinook salmon (*Oncorhynchus tshawytscha*) structured of 96 full-sib families nested within 30 half-sib families; 2) determined the genetic and environmental components of variation in body morphometry of these fish at the parr-smolt stage of development (Hard et al. in press); 3) released to sea 257,093 of these fish, each identified with full-sib family-specific coded-wire tags; 4) cultured several hundred 2-, 3-, and 4-year-old PIT-tagged fish from the same cohort to maturity in marine netpens; 5) established 300 1997-brood PIT-tagged smolts from a total of 27 families of 3-year-old captive adults in 3 experimentally inbred lines in seawater netpens; and 6) established 400,000 1998-brood embryos from a total of 100 families of 4-year-old hatchery adults in 3 experimentally inbred lines. The experimentally inbred lines correspond to a minimal increment in inbreeding (randomly mated control), a moderate increment in inbreeding (half-sib parents, corresponding to an approximate increase in inbreeding of 12.5%), and a substantial increment in inbreeding (full-sib parents, corresponding to an approximate increase in inbreeding of 25%). We expect to have preliminary data on the effects of one generation of inbreeding as early as spring 1999, when inbred offspring are transferred to seawater.

Due to the multiple generations this experiment requires to implement substantial inbreeding, we anticipate that the study will continue for at least the next five years. In FY2000 we shall continue the experiment in an attempt to address our stated hypotheses. We shall evaluate whether juvenile fish in the three experimentally inbred lines (randomly mated, half-sib mated, and full-sib mated) differ in stage-specific survival, growth, and development rate after one generation of inbreeding in their parents. We will be able to make this evaluation in the captively reared 1997- and 1998-brood fish to the post-smolt stage in seawater netpens. Subsequent evaluations will focus on survival to adulthood and life history characteristics of fish maturing in the captively reared groups and in the adults returning from the hatchery releases.

We shall use analyses of family variance in survival and growth within and among lines to test the hypotheses stated above and evaluate the effects of inbreeding on these characters, following methods developed by Lynch (1988) and Lynch and Walsh (1998). If necessary, we shall follow these tests with retrospective power analyses to shed light on the sorts of differences that would be necessary to detect differences (Cohen 1988).

In addition, we will analyze the following data for five-year-old adults maturing in 1999 and returning to Grovers Creek Hatchery: survival, body length and weight, morphometry (via digitized photographs), and for females, egg size and fecundity. We will continue to collect size and growth information on captively reared 1997-brood individuals at approximately quarterly intervals. If sufficient fish are available, we plan to analyze data obtained on morphometry of approximately 20 1997-brood offspring sampled from each full-sib family in each experimental group to evaluate first-generation effects of inbreeding, if any, on morphology. We will continue to remove and, when feasible, decode coded-wire tags from fish sampled as part of the vibriosis challenge experiment conducted in 1995. Collectively, these data will provide a comparison of first-generation inbreeding effects on early life-history traits and, through examination of the control population, an assessment of relative environmental influences in consecutive generations on these traits.

g. Facilities and equipment

Office space, hatchery, and laboratory facilities at the following locations are being utilized to conduct the proposed and ongoing research: Northwest Fisheries Science Center (NWFSC), Seattle, WA, NMFS Manchester Marine Field Station, Manchester, WA; USGS, Northwest Biological Science Center, Seattle, WA; School of Fisheries, University of Washington; University of Idaho Haggerman Fish Culture Experiment Station. Laboratories have all necessary equipment for large scale analysis of samples for endocrine, genetic, nutrition and fish pathology studies. The fish holding facilities are adequate for all proposed studies. Construction of unique facilities for behavioral observation of adult salmon spawning and juvenile fish have been completed at the Manchester Marine Field Station.

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h. Budget

see table

Section 9. Key personnel

PENNY SWANSON Ph.D., Project Investigator, Fish Reproductive Physiology
 Duties: project administration, oversee and conduct research in reproductive physiology of salmonid fish especially related to growth and reproduction.
 Expertise: reproductive physiology of fish, particularly endocrine control of reproduction, pituitary hormone biochemistry
 Work Address: Northwest Fisheries Science Center
 National Marine Fisheries Service
 2725 Montlake Blvd. East
 Seattle, WA 98112
 ph. 206-860-3282, Fax. 206-860-3267

email: penny.swanson@noaa.gov

Education

University of Washington Seattle, WA	B.A., Summa Cum Laude	1978	Zoology
University of Wisconsin and Madison, WI	M.S.	1980	Endocrinology Reproductive Physiology
University of Washington Seattle, WA	Ph.D.	1986	Zoology

Employment

1992-present Physiologist, National Marine Fisheries Service, Seattle.

Aff. Asst. Professor, School of Fisheries, Univ. Washington, Seattle

1990-1992 Physiologist, (50 %) National Marine Fisheries Service, Seattle.

Fish Biologist IV (50%), School of Fisheries, University of Washington, Seattle.

1987-1990 Post-docotoral Research Associate, School of Fisheries, University of Washington, Seattle.

Supervisor: Dr. W. W. Dickhoff

1986-1987 Research Fellow, Japanese Society for Promotion of Science, School of Fisheries Sciences, Kitasato University, Sanriku, Japan.

Supervisor: Dr. H. Kawauchi

1986 Teaching Assistant, Dept. of Zoology, University of Washington, Seattle.

Course: Reproductive Endocrinology of Vertebrates

1985-1986 Research Assistant, Dept. of Zoology, University of Washington, Seattle.

Advisor: Dr. A. Gorbman

1984-1985 NIH Trainee, Molecular and Cellular Biology Training Program, University of Washington, Seattle. Advisor: Dr. A. Gorbman

1982-1984 Teaching Assistant, Dept. of Zoology, University of Washington, Seattle.

Courses: Comparative Anatomy; Comparative Physiology

1980-1982 Research Specialist, Aquaculture Research Laboratory, University of Wisconsin, Madison. Supervisor: Dr. T. Kayes

1978-1980 NIH Trainee, Endocrinology and Reproductive Physiology Program, University of Wisconsin, Madison. Advisors: Dr. R. Bremel and Dr. J. Gorski

Representative publications

Swanson, P., Suzuki, K., Kawauchi, H. and Dickhoff, W. W. (1991). Isolation and characterization of coho salmon gonadotropins: GTH I and GTH II. Biol. Reprod. 44:29-38.

Swanson, P.(1991). Salmon gonadotropins: Reconciling old and new ideas. In, "Reproductive Physiology of Fish" (A.P. Scott and J.P. Sumpter, eds.), pp.2-7. Univ. of East Anglia, Norwich, England.

Oppen-Berntsen, D.O., Olsen, S.O., Rong, C.J., Tarranger, G.L., Swanson, P., and Walther, B.T. (1994). Plasma levels of eggshell ZR-proteins, estradiol-17 α , and gonadotropins during an annual reproductive cycle of Atlantic salmon (*Salmo salar*). J. Exp. Zool. 268:59-70.

Planas, J.V. and Swanson, P. (1995). Maturation-associated changes in the response of the salmon testis to the steroidogenic actions of gonadotropins (GTH I and GTH II). *Biol. Reproduction* 52:697-704.

Planas, J. V., Goetz, F. W., and Swanson, P. 1997. Stimulation of brook trout ovarian steroidogenesis by gonadotropins (GTH I and GTH II) is mediated by protein kinase A. *Biol. Reprod.* 57:647-654.

JEFF HARD, Ph.D., Project Investigator, Fish Genetics

Northwest Fisheries Science Center

Conservation Biology Division

2725 Montlake Blvd. E.

Seattle, WA 98112

206/860-3275

jeff.hard@noaa.gov

EDUCATION

1979, B.S. Biology, Oregon State University

1984, M.S. Fisheries, University of Alaska

1991, Ph.D. Ecology and Evolutionary Biology, University of Oregon

EMPLOYMENT HISTORY

1992-present Fishery Research Biologist, U.S. Department of Commerce, NMFS, Northwest Fisheries Science Center, Seattle, Washington

1986-91 Graduate Fellow, Department of Biology, University of Oregon, Eugene

1982-86 Fishery Research Biologist, U.S. Department of Commerce, NMFS, Auke Bay Laboratory

1977-82 Fishery Technician, U.S. Department of Commerce, NMFS, Auke Bay Laboratory

1975-76 Research Assistant, Institute of Marine Science, University of Alaska, Fairbanks, Alaska, and CEPEX (Controlled Ecosystem Pollution EXperiment) Project, Patricia Bay Lab., Sidney, B.C., Canada

REPRESENTATIVE PUBLICATIONS

Berejikian, B. A., E. P. Tezak, S. L. Schroder, C. M. Knudsen, and J. J. Hard. In press. Reproductive behavioral interactions between wild and captive reared coho salmon (*Oncorhynchus kisutch*). *ICES Journal of Marine Science*.

Hard, J. J., R. G. Kope, and W. S. Grant. In press. Review of the status of pink salmon from Washington, Oregon, and California. In D. MacDonald, C. Steward, and J. Williams (editors), *Sustainable Fisheries Management*, Ann Arbor Press, Ann Arbor, MI.

Hard, J. J. 1995. A quantitative genetic perspective on the conservation of intraspecific diversity. In J. L. Nielsen (editor), *Evolution and the aquatic ecosystem: defining unique units in population conservation*, p. 304-326. *American Fisheries Society Symposium* 17, Bethesda, Maryland.

Hard, J. J. 1995. Genetic monitoring of life-history characters in salmon supplementation: problems and opportunities. In R. G. Piper and H. L. Schramm

(editors), Uses and effects of cultured fishes in aquatic ecosystems, p. 212-225. American Fisheries Society Symposium 15, Bethesda, Maryland.
Hard, J. J., R. P. Jones, Jr., M. R. Delarm, and R. S. Waples. 1992. Pacific salmon and artificial propagation under the Endangered Species Act. U.S. Department of Commerce, NOAA Technical Memorandum NMFS-NWFSC-2, 56 p.

LEE HARELL, DVM, Project Investigator, Fish Pathology

Address:

National Marine Fisheries Service
Resource Enhancement and Utilization Technology
Manchester Marine Experimental Station
Box 130
Manchester. WA. 98353

Education:

B.S. (Animal Husbandry). College of Agriculture, University of Florida, Gainesville, FL. 1960.

D.V.M. (Veterinary Medicine). School of Veterinary Medicine, Auburn University, Auburn, AL. 1964.

M.S. (Fisheries Biology). School of Fisheries, University of Washington, Seattle, WA. 1973.

Expertise:

Practice of fish medicine and parasitology. Prevention and therapy of infectious and parasitic diseases of marine and freshwater fishes.

Representative Publications:

Harrell, L. W., and T. L. Deardorff. 1990. Human nanophyetiasis: transmission by handling naturally infected coho salmon (*Oncorhynchus kisutch*). J. Inf. Dis. 161(1):146-148.

Flagg, T. A. and L. W. Harrell. 1990. Use of water-to-water transfers to maximize survival of salmonids stocked directly into seawater. Prog. Fish Cult. 52:127-129.

Harrell, L. W. and T. M. Scott. 1985. *Kudoa thyrsitis* (Gilchrist) (Myxosporea, Multivalvulidae) in Atlantic salmon *Salmo salar*. J. Fish Disease 8:324-332.

L. W. Harrell, R. A. Elston, T. M. Scott, and M. T. Wilkinson. 1986. A significant new systemic disease of net-pen reared chinook salmon *Oncorhynchus tshawytscha* brood stock. Aquaculture 55:249-262.

R. A. Elston, L. W. Harrell, and M. T. Wilkinson. 1986. Isolation and in vitro characteristics of chinook salmon *Oncorhynchus tshawytscha* rosette agent. Aquaculture 56:1-21.

KARL SHEARER, Ph.D., Project Investigator, Fish Nutrition

Education:

University of Washington, 1971, B. S., Fisheries Science

University of Washington, 1986, M. S., Fisheries Science

University of Bergen, 1991, Ph. D., Fisheries Biology

Employment:

1980-present	Research Biologist, NMFS, Seattle, WA.
1978-1980	Research Biologist, DØmsea Farms, Bremerton, WA.
1978	Fisheries Biologist III, WA State Game Dept., Olympia, WA.
1973-1978	Biologist-In-Charge, Inland Fish. Res. Stn., NSW, Australia.
1972-1973	Research Fisheries Biologist, NSW, Australia.
1971	Biologist, Washington Water Power, Spokane, WA.

Research Interests:

Utilization of plant proteins in salmonid diets, pigmentation of salmonids, appetite regulation, bioenergetics and salmonid life cycle, dietary nutrient requirements.

Selected Publications:

Shearer, K. D., Åsgård, T., Andorsdóttir, G. and Aas, G. H. (1993). Whole body proximate and elemental composition of Atlantic salmon (*Salmo salar*) during the life cycle. *J. Fish. Biol.*, 44: 785-797.

Shearer, K. D. (1994) Factors affecting the proximate composition of cultured fishes with emphasis on salmonids. *Aquaculture*. 119: 63-88.

Shearer, K. D. (1995) The use of factorial modeling to determine the dietary requirements for essential elements in fish. *Aquaculture*. 133: 57-72.

Shearer, K. D., Silverstein, J. T. and Plisetskaya, E. M. (1997) The role of adiposity in food intake control of juvenile chinook salmon (*Oncorhynchus tshawytscha*). *Comp. Biochem. Physiol.*, 118A: 1209-1215.

Shearer, K. D., Silverstein, J. T. and Dickhoff, W. W. (1997) Manipulation of growth and adiposity of juvenile chinook salmon. *Aquaculture*. 157: 311-323.

BARRY BEREJIKIAN, Ph.D, Project Investigator- Fish Behavior
National Marine Fisheries Service

Education

California Polytechnic State University, San Luis Obispo, Bachelor of Science, 1990
University of Washington, Master of Science, 1992
University of Washington, Ph.D., 1995

Expertise

Dr. Berejikian has been conducting scientific research on the effects of artificial culture on the behavior of salmonids since 1992. Beginning in 1995, Dr. Berejikian has lead a multi-year, multi-disciplinary, cooperative research effort between NMFS and the Washington Department of Fish and Wildlife to evaluate the relative reproductive success of captively-reared and wild salmon. He has annually conducted intense monitoring of chinook and coho salmon reproductive ecology to complete reproductive behavior experiments. This work has already produced one refereed journal article, with two other manuscripts currently under internal review. Dr. Berejikian has also worked

closely with NMFS geneticists to develop and apply new DNA "fingerprinting techniques" to directly assess the reproductive success of spawning captively reared and wild salmon. The information generated from these studies has, and will continue to help guide development of captive broodstock strategies for use in recovery of salmon in the Snake River and Columbia River basins.

Publications

Berejikian, B. A. 1995. The effects of hatchery and wild ancestry and experience on the relative ability of steelhead trout fry (*Oncorhynchus mykiss*) to avoid a benthic predator. *Can. J. Fish. Aquat. Sci.* 52:2476-2482.

Berejikian, B. A., S. B. Mathews, and T. P. Quinn. 1996. The effects of hatchery and wild ancestry and rearing environment on the development of agonistic behavior in steelhead trout (*Oncorhynchus mykiss*) fry. *Can. J. Fish. Aquat. Sci.* 53:2004-2014

Berejikian, B. A., E. P. Tezak, S. L. Schroder, C. M. Knudsen, and J. J. Hard. In press. Reproductive behavioral interactions between spawning wild and captively reared coho salmon (*Oncorhynchus kisutch*). *ICES Journal of Marine Science*.

MICHAEL B. RUST

20220 29th Ave SE, Bothell, Washington 98012 USA

Home: (206) 483-4155, Office: (206) 860-3382, Fax (206) 860-3394

Internet - mike.rust@noaa.gov

Education:

BA Biology. University of Colorado at Boulder, 1980.

MS Animal Science. University of California at Davis, 1987.

MS International Agricultural Development. Univ. California at Davis, 1987.

Ph D Fisheries. University of Washington, Seattle, 1995.

Employment:

Research Fishery Biologist, National Marine Fisheries Service, Resource Enhancement and Utilization Technology Division, Northwest Fisheries Science Center, Seattle, WA 98112, March 1994- Present

Visiting Scientist, Institute of Marine Research, Austevoll Aquaculture Research Station, N-5392 Storebø, Norway, March 1995 - July 1995.

Teaching Assistant, School of Fisheries, University of Washington, Seattle, WA 98195, March 1994 - June 1994.

Research Associate, School of Fisheries, University of Washington, Seattle, WA 98195, November 1989 - March 1994.

Scientist/Consultant, Aquaresearch Ltd., North Hatley, Québec, Canada, June 1988 - July 1989.

Chief Scientist for Haiti Project, Caribbean Marine Research Center, Cap Haitien, Haiti, March 1987 - May 1988.

Aquaculture Projects Coordinator, University of California at Davis, Sustainable Agriculture Program, April 1985 - March 1987.

Research Assistant, University of California at Davis, Department of Animal Science, January 1985 - March 1987.

Peace Corps Volunteer, Talibon District Fisheries Office, Talibon, Bohol,

Philippines, June 1980 - July 1982.

Expertise: Developmental biology, nutrition and engineering related to fish. Primary expertise is in the development of the digestive system in fish; larval and broodstock nutrition; and live and microparticulate feeds for first-feeding fish.

Selected Publications

Ehrlich, K. F., M. C. Cantin, M. B. Rust and B. Grant. 1989. Growth and survival of larval and post larval smallmouth bass fed a commercially prepared dry feed and/or *Artemia* nauplii. *Journal of the World Aquaculture Society*, Vol 20:1, pp. 1-6.

Rust, M. B., Hardy, R. W., and Stickney, R. R., 1993. A new method for force-feeding larval fish. *Aquaculture*. 116: 341-352.

Rust, M. B. 1995. Quantitative aspects of nutrient assimilation in six species of fish larvae. Ph. D. Dissertation, University of Washington, Seattle, 150 p.

Scott, T. M., and M. B. Rust. 1996. A computer automated cold water recirculating system for aquaculture research. In: G. S. Libey and M. B. Timons (eds) *Successes and Failures in Commercial Recirculation Aquaculture*. Northeast Regional Agricultural Engineering Service, NRAES-98, pp 562-574.

Rust, M. B., and F. T. Barrows. Submitted. An image analysis approach to determine microparticulate feed acceptability with larval fish. *Proceedings of the symposium on Marine Finfish and Shellfish Aquaculture, Marine Stock Enhancement, and Open Ocean Engineering and the 26th UJNR aquaculture panel meeting*, September 16-18, 1997.

RONALD JAMES PASCHO, principal investigator

Institution: Western Fisheries Research Center, Seattle, WA

Title: Research Microbiologist, GS-13

Current Responsibilities: Project leader and principal investigator for fish health research projects, particularly those involving immunological and bacteriological procedures.

Education

M.S., School of Fisheries, University of Washington, 1993

B.S., Microbiology, University of Washington, 1975

A.A., Shoreline Community College, Seattle, WA, 1972

Recent Positions Held

Research Microbiologist, Project Leader, Bacteriology Section, National Fisheries Research Center-Seattle, USFWS, 1983-present

Research Microbiologist, Virology Section, National Fisheries Research Center-Seattle, USFWS, 1976-1983

Expertise

Ronald Pascho's major areas of research have focused on the development and application of new detection methods in the study of important pathogenic microorganisms of Pacific salmon. He is currently involved in a program to investigate whether the nonspecific host defenses of sockeye salmon (*Oncorhynchus nerka*) modulate with seasonal and lifestage changes, or differing rearing conditions.

Relevant Publications

- Pascho, R.J., D. Chase, and C.L. McKibben. 1998. Comparison of the membrane-filtration fluorescent antibody test, the enzyme-linked immunosorbent assay, and the polymerase chain reaction to detect *Renibacterium salmoninarum* in salmonid ovarian fluid. *Journal of Veterinary Diagnostic Investigation* 10:60-66.
- Pascho, R.J., T.D. Goodrich, and C.L. McKibben. 1997. Evaluation by enzyme-linked immunosorbent assay (ELISA) of *Renibacterium salmoninarum* bacterins affected by persistence of bacterial antigens. *Journal of Aquatic Animal Health* 9:99-107.
- Pascho, R.J., D.G. Elliott, and S. Achord. 1993. Monitoring of the in-river migration of smolts from two groups of spring chinook salmon (*Oncorhynchus tshawytscha*) with different profiles of *Renibacterium salmoninarum* infection. *Aquaculture and Fisheries Management* 24:163-169.
- Pascho, R.J., D.G. Elliott, and J.M. Streufert. 1991. Brood stock segregation of spring chinook salmon *Oncorhynchus tshawytscha* by use of the enzyme-linked immunosorbent assay (ELISA) and the fluorescent antibody technique (FAT) affects the prevalence and levels of *Renibacterium salmoninarum* infection in progeny. *Diseases of Aquatic Organisms* 12:25-40.

MARK STROM

National Marine Fisheries Service, Northwest Fisheries Science Center, Resource Enhancement and Utilization Technologies Division

Education

B.S. Microbiology, 1977, University of Washington, Seattle
 M.S. Microbiology, 1982, University of Washington, Seattle
 Ph.D. Microbiology, 1992, University of Washington, Seattle

Expertise

Dr. Strom has been conducting research on mechanisms of virulence and pathogenicity of bacteria since the early 1990s. He now leads a team of seven staff working on bacterial pathogens of fish and humans. Dr Strom is known internationally, and has been invited often to lecture at scientific meetings around the world. In 1998 he began working on the fish health portion of the captive broodstock research, and he brings an excellent background and experience in experimental design and data analysis to the project.

Selected publications:

- Strom, M. S., H. C. Douglas, and C. A. Evans. 1982. Stimulation of the growth of cutaneous strains of *Peptococcus saccharolyticus* by iron, haematin and blood. *J. Gen. Microbiol.* 128:387-391.
- Evans, C. A., J. R. Crook, and M. S. Strom. 1984. The bacterial flora of the forehead and back of Alaskan Native villagers in summer and in winter. *J. Invest. Dermatol.* 82:294-297.
- Strom, M. S., M. W. Eklund, and F. T. Poysky. 1984. Plasmids in *Clostridium botulinum* and related *Clostridium* species. *Appl. Environ. Microbiol.* 48:956-963.
- Strom, M. S., D. Nunn, and S. Lory. 1991. Multiple roles of the pilus biogenesis protein PilD: Involvement of PilD in excretion of enzymes from *Pseudomonas aeruginosa*. *J. Bacteriol.* 173:1175-1180.
- Strom, M. S., D. N. Nunn, and S. Lory. 1993. A single bifunctional enzyme, PilD, catalyzes cleavage and N-methylation of proteins belonging to the type IV pilin family. *Proc. Natl. Acad. Sci.* 90:2404-2408.

- Strom, M. S., and S. Lory. 1993. Structure-function and biogenesis of the type IV pili. *Ann. Rev. Microbiol.* 47:565-596.
- Pepe, C. M., M. W. Eklund, and M. S. Strom. 1996. Cloning of an *Aeromonas hydrophila* type IV pilus biogenesis gene cluster: complementation of pilus assembly functions and characterization of a type IV leader peptidase/N-methyltransferase required for extracellular protein secretion. *Mol. Microbiol.* 19:857-869.
- Lory, S. and M. S. Strom. 1997. Structure and function relationship of type IV prepilin peptidase of *Pseudomonas aeruginosa*. *Gene* 192:117-121.
- Rhodes, L. D., W. B. Nilsson, and M. S. Strom. 1998. Sensitive detection of *Renibacterium salmoninarum* in whole fry, blood, and other tissues of Pacific salmon by Reverse Transcription-PCR. *Molecular Marine Biology and Biotechnology*. In Press.

Section 10. Information/technology transfer

Information generated by this project will be communicated by annual reports, publication in peer-reviewed scientific journals, presentations at workshops and scientific conferences, and regular communication with and participation in Technical Oversight Committees for the ongoing captive broodstock programs.

Congratulations!